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ERRATA

Page 179, table III, second column heading, for ''Average,'' read Total.
Cover page four, April number, sixth title, for ''*Photoperiod*'' read *Photoperiodic*.
Page 415, author's initial, for ''B'' read S.
Page 434, in the regression equation, for ''1000'' read 10000.
Page 435, in both regression equations, for ''1000'' read 10000.
Cover page four, July number, first title, for ''*on distribution*'' read *on nitrogen dis-
    tribution*.
Cover page four, July number, first author's initial, for ''B'' read S.

THE PROCESS OF OIL FORMATION AND ACCUMULATION IN THE MACADAMIA[1]

WINSTON W. JONES AND LILY SHAW

In previous investigations of the problem of oil formation in the pecan it has been shown (**4, 16**) that the fatty acids, and consequently the oil and fats, are formed from carbohydrates, either stored or currently synthesized. The present paper deals with formation and changes in characteristics of the oil in the Macadamia from the young embryo to the mature fruit.

The oil present in the mature Macadamia nut (*Macadamia ternifolia* F.v.M. var. *integrifolia* Maiden) is very similar to pecan oil (**2**), consisting largely of the glyceride of oleic acid with small amounts of the glycerides of palmitic, stearic, and myristic acids (**3**). It has a saponification value of 197.3, an acid value of 0.57, and an iodine number of 75.2. As pointed out above, carbohydrates provide the principal carbon source from which fats are formed, but M'CLENAHAN (**9**) believed that in the case of black walnuts the oil was derived from tannins; SCURTI and TOMMASI (**13**) thought that the higher alcohol, oleanol, was converted into oil in the olive.

The formation of limited amounts of fatty acids from hexoses outside of the living cell has been accomplished by SMEDLEY (**14**) who prepared these acids by first breaking down hexoses into C_4 compounds followed by oxidation of the latter into pyruvic acid which was then condensed into acids with longer carbon chains. It is also of interest to note that fatty acids are prepared commercially from the paraffin hydrocarbons (**5**).

Materials and methods

All plant material herein described was collected on June 1, 1940, from an orchard near Honolulu, T. H. The fruiting habits of the Macadamia are such that at this season of the year nuts of all ages from very young to mature can be collected from a single tree. The samples collected from each of six seedling trees were brought to the laboratory and the nuts from each tree divided into five age groups based on known condition of shell as related to age (**7**). Each sample was composed of about one hundred and forty 90-day-old fruits and 35 fruits from each subsequent age group from each

of six trees. Since the carbohydrate and ether extract contents of the
Macadamia husk and shell change very little during their development (7),
these portions of the fruits were discarded and only embryos were studied.
A description of the fruits at the time of harvest, including the approximate
number of days from flowering for each age group and the average fresh
weight per embryo, is given in table I.

TABLE I

CONDITION OF MACADAMIA FRUITS OF DIFFERENT AGE GROUPS AT TIME OF SAMPLING

AGE GROUP	TIME AFTER FLOWERING	AVERAGE FRESH WEIGHT PER EMBRYO	APPEARANCE AND TEX- TURE OF SHELL
	days	*gm.*	
1	90	1.14	Soft and white
2	111	2.81	Hard and white
3	136	3.41	Hard and light brown
4	185	2.88	Hard and brown
5	215	2.78	Hard and dark brown

DRY WEIGHT

The youngest samples were dried at 100° C. for 2 hours in an air oven
and then in a vacuum oven at 80° C. for 48 hours. Older samples required
8 to 24 hours longer in the air oven adjusted to 80° C. On removal from
the vacuum oven the embryos were cooled over calcium chloride and dry
weights obtained after which the material was chopped into small particles
by means of a sharp knife, redried and returned to the desiccator and held
until analyzed.

ANALYTICAL METHODS

OIL.—The redried, finely chopped material was removed from the desic-
cator and about 2-gram aliquots, the exact weights of which were recorded,
were weighed into alundum cups and extracted with anhydrous petroleum
ether in a continuous drip extractor for 6 hours. The sample was then
removed, reground in an agate mortar, and re-extracted until free of oil.
The petroleum ether was removed, and the extract dried, weighed and
reported as oil. The oil was then held in the desiccator for the determina-
tion of oil characteristics.

SUGARS.—The sugars in the oil-free residue were extracted with 80 per
cent. alcohol. An aliquot of the extract was freed of alcohol, cleared with
neutral lead acetate and potassium oxalate, and reducing sugars determined
by the method of STILES, PETERSON, and FRED (15). After treatment with
invertase, sucrose was determined.

NITROGEN.—Soluble nitrogen was determined on aliquots of the alcoholic
extract by the reduced iron method of PUCHER, LEAVENWORTH, and VICKERY
(11) adapted to the micro-Kjeldahl method described by PREGL (10). In-
soluble nitrogen was determined on the residue of the alcoholic extraction
by the above micro-method.

ACID-HYDROLYZABLE MATERIALS.—These were obtained from an aliquot of the residue from the alcoholic extraction by determining the amount of reducing substances formed after digestion with 2.5 per cent. HCl under a reflux condenser for three hours.

OIL CHARACTERISTICS.—The ether extract was analyzed according to the official methods of the Association of Official Agricultural Chemists (1) with the exception of hydroxyl number determinations which were obtained by the sealed tube method of ROBERTS and SCHUETTE (12).

Results and discussion

The data are presented in two parts: (1) those having to do with the accumulation of oil, carbohydrates, and nitrogen; and (2) those having to do with the analysis of the ether extracts.

ACCUMULATION OF OIL, CARBOHYDRATES, AND NITROGEN

The accumulation of dry matter in the Macadamia embryo (table II) is

TABLE II

DRY WEIGHT CHANGES IN THE MACADAMIA FRUIT

AGE GROUP	TIME AFTER FLOWERING	AVERAGE DRY WEIGHT PER EMBRYO	FRESH WEIGHT PER EMBRYO	PERCENTAGE DRY WEIGHT
	days	gm.	gm.	%
1	90	0.067	1.138	5.0
2	111	0.491	2.806	17.5
3	136	0.992	3.410	29.1
4	185	1.382	2.877	48.0
5	215	1.882	2.781	67.7

continuous from the time of the first sample through to maturity. This is not the case for fresh weight where the peak is attained rather early in the life of the fruit with a consequent slow drying of the embryo so that at the time of maturity the dry material consists of 67.7 per cent. of the total weight of the embryo.

The data on the accumulation of oil, carbohydrates, and nitrogen are shown in tables III and IV. The results presented in tables III and IV agree closely with those already published (7, 8); they will be discussed more fully in connection with the formation of oil.

The Macadamia plant, for the first 90 days after flowering, is largely occupied with the laying down of structures of the fruit and the growth of the husk, shell, and endosperm. Later the embryo enlarges rapidly and begins the formation and accumulation of oil. During the period from 90 to 185 days after flowering, 44 per cent. of the total growth period of the fruit, 70 per cent. of the total oil is formed. It is of interest to note that during the accumulation of oil, protein and sucrose also accumulated.

TABLE III

CARBOHYDRATES, NITROGEN, AND OIL CHANGES IN THE MACADAMIA EMBRYO, EXPRESSED
AS PERCENTAGE OF DRY WEIGHT, IN RELATION TO AGE

CONSTITUENT	DAYS AFTER FLOWERING				
	90	111	136	185	215
	%	%	%	%	%
Reducing sugar	1.47	3.21	1.07	0.41	0.30
Sucrose	6.07	24.07	21.91	9.19	5.50
Total sugar	7.54	27.28	22.98	9.60	5.80
Soluble nitrogen	2.92	1.13	0.61	0.33	0.27
Insoluble nitrogen	1.96	1.91	1.58	1.39	1.43
Total nitrogen	4.88	3.04	2.19	1.72	1.70
Acid-hydrolyzable matter	4.54	4.88	3.85	2.56	2.16
Soluble solids in 80 per cent. alcohol	60.10	39.92	28.36	14.82	9.88
Ether and alcohol insoluble material	36.43	28.88	23.69	17.89	16.68
Petroleum ether extract	3.46	31.19	47.94	67.28	73.44

ANALYSIS OF ETHER EXTRACT

Table V shows the changes in the oil characteristics during the development of the embryo.

It is clear from table V that the most marked changes in the oil characteristics occur during the 21-day period from 90 to 111 days after flowering. During this period the acid number drops from 163.9 to 6.56 with a final value of 0.57; the saponification number drops from 380.6 to 204.2 with a final value of 197.3; soluble acids drop from 23.65 to 1.67 per cent. with a final value of 0.26 per cent.; and the iodine number increases from 64.4 to 75.4 with a final value of 75.2. The unsaponifiable material is never above one per cent., indicating that the ether extract consists largely of fatty acids or esters. It is apparent that in the early stages of oil formation

TABLE IV

CARBOHYDRATE, NITROGEN, AND OIL CHANGES IN THE MACADAMIA EMBRYO, EXPRESSED
AS MILLIGRAMS PER EMBRYO, IN RELATION TO AGE

CONSTITUENT	DAYS AFTER FLOWERING				
	90	111	136	185	215
	mg.	mg.	mg.	mg.	mg.
Reducing sugar	0.85	13.69	8.39	5.41	3.97
Sucrose	3.93	115.03	196.65	128.25	100.87
Total sugar	4.78	128.72	205.04	133.66	104.84
Soluble nitrogen	1.94	5.26	5.33	4.33	4.75
Insoluble nitrogen	1.33	8.28	14.35	19.08	26.12
Total nitrogen	3.27	13.54	19.68	23.41	30.87
Acid-hydrolyzable matter	3.00	23.27	34.34	33.43	39.41
Soluble solids in 80 per cent. alcohol	40.02	187.25	251.65	201.20	177.72
Ether and alcohol insoluble material	24.92	139.29	213.54	245.12	304.22
Petroleum ether extract	2.40	164.95	456.81	936.02	1339.39

short-chain fatty acids accumulate as such and these are not esterified until somewhat later. While the accumulation of oil in its final form tends to obscure the actual process of formation the data presented shed some light on the change from carbohydrates to fats.

In the Macadamia it is likely that carbohydrates are the most important source of the oil. Microchemical tests have shown that flowering branches contain considerable quantities of stored starch while non-flowering branches on the same tree often contain no trace of starch. Thus there are available two sources of carbohydrates: stored starch, and that from current photosynthesis. The starch in the flowering branches, however, largely disappears by the time oil formation begins in the developing embryo, which in the Macadamia is the organ of oil formation and storage (6, 8). No starch has ever been found in the embryo during development, and oil formation

TABLE V

CHANGES IN THE CHARACTERISTICS OF MACADAMIA OIL IN RELATION TO AGE

CHARACTERISTICS	DAYS AFTER FLOWERING				
	90	111	136	185	215
Acid number	163.90	6.56	2.26	0.73	0.57
Saponification number	380.6	204.2	199.7	197.7	197.3
Soluble acids (per cent.)	23.65	1.67	0.46	0.23	0.26
Insoluble acids (per cent.)	48.14	81.49	86.42	94.09	94.21
Iodine number	64.4	75.4	74.4	75.7	75.2
Unsaponifiable matter (per cent.)	*	0.75	0.58	0.42	0.34
Hydroxyl number		11.1	9.0	6.0	4.8
Index of refraction, 25° C.		1.4681	1.4669	1.4658	1.4657

* Insufficient material for analysis.

necessitates the conversion of the hexoses and/or sucrose that are moved into the embryo into fatty acids and glycerol for the formation of the glycerides.

The movement of materials (soluble carbohydrates and soluble nitrogen) into, and the accumulation of non-mobile materials (oil and protein) in, the Macadamia embryo proceed rapidly as the embryo enlarges. Since the embryo at the onset of rapid accumulation consists of only a few cells, and since the husk and shell change very little in composition (7), this material undoubtedly moves into the fruit from outside sources. Aside from the mineral constituents and soluble nitrogen compounds, this material most likely was moved into the embryo as carbohydrates, probably hexose sugars (4). If this is the case, the hexose sugars are immediately transformed into other compounds as there is never found any accumulation of reducing sugars.

In a study of the oil characteristics of Macadamia, the accumulation of glycerides obscures the early steps in oil formation since a large percentage of the ether extract consists of these "fully formed" glycerides. Thus the process of oil formation might appear to be somewhat different in the early

stages as compared to the later stages; this is not necessarily true, however, since the process may be masked by the fully formed glycerides. At first there is a marked accumulation of free fatty acids of low molecular weight with a comparatively low iodine number, which rapidly disappear as esterification begins, accompanied by a drop in the saponification value, indicating the presence of a much longer chain fatty acid.

The accumulation of the free fatty acids can be explained in one of two ways: (a) either the formation of glycerol begins after the formation of the acids, or (b) the enzymes necessary for the formation of esters are not present. In the early stages the degree of unsaturation is low and hydroxyl acids are present; later, concomitant with oil formation, the degree of saturation increases and the free acids largely disappear.

Summary

1. Beginning at approximately 90 days after flowering of Macadamia, embryo enlargement and accumulation of oil are very rapid; 70 per cent. of the oil is formed in 44 per cent. of the total growth period of the fruit.

2. The material utilized for oil formation moves into the fruit from outside sources. Oil synthesis and protein synthesis occur simultaneously.

3. In the early stages of oil synthesis short chain saturated fatty acids are formed first and accumulate; later, long chain unsaturated fatty acids are formed.

4. Hexose sugar is apparently the starting point for oil formation. From the sugar, short chain saturated fatty acids are formed. The short chain acids are converted into long chain unsaturated fatty acids as synthesis proceeds.

5. In the early stages of oil formation the short chain acids and glycerol do not combine probably because of the absence of the proper esterase.

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LITERATURE CITED

1. Association of Official Agricultural Chemists. Official and tentative methods of analysis. Fourth ed. Washington, D. C., 1935.

2. BOONE, PAUL D. Chemical constituents of pecan oil. Jour. Ind. & Eng. Chem. 16: 54–55. 1924.

3. CHEEL, EDWIN, and MORRISON, F. R. The cultivation and exploitation of the Australian nut (*Macadamia ternifolia*, F.v.M., and *Macadamia integrifolia*, Maiden et Betche). Technol. Museum, Sydney, Australia, Bull. 20. 1935.

4. FINCH, A. H., and VAN HORN, C. W. The physiology and control of pecan nut filling and naturity. Arizona Agr. Exp. Sta. Tech. Bull. 62. 1936.

5. GROGGINS, P. H. Unit processes in organic synthesis. McGraw-Hill Book Co., Inc. New York. 1938.

6. HARTUNG, M. E., and STOREY, W. B. The development of the fruit of *Macadamia ternifolia*. Jour. Agr. Res. **59**: 397–406. 1939.

7. JONES, WINSTON W. The physiology of oil production in the macadamia (*Macadamia integrifolia*, Maiden et Betche). Proc. Amer. Soc. Hort. Sci. **35**: 239–245. 1937.

8. ————————. A study of developmental changes in composition of the macadamia. Plant Physiol. **14**: 755–768. 1939.

9. M'CLENAHAN, F. M. The development of fat in the black walnut. Jour. Amer. Chem. Soc. **31**: 1093–1098. 1909.

10. PREGL, FRITZ. Quantitative organic micro-analysis. P. Blakiston's Son and Co., Inc., Philadelphia. 1928.

11. PUCHER, G. W., LEAVENWORTH, C. S., and VICKERY, H. B. Determination of total nitrogen of plant extracts in presence of nitrates. Ind. & Eng. Chem. Anal. Ed. **2**: 191–193. 1930.

12. ROBERTS, WILLARD L., and SCHUETTE, H. A. Determination of hydroxyl number of oil, fats, and waxes. An accurate rapid method. Ind. & Eng. Chem. Anal. Ed. **4**: 257. 1932.

13. SCURTI, F., and TOMMASI, G. In regard to the formation of fatty acid in oleaginous fruits. Rend. Soc. Chim. Ital. **3**: 358–363. 1912.

14. SMEDLEY, I. The biochemical synthesis of fatty acids from carbohydrates. Jour. Physiol. **45**: 25–27. 1912.

15. STILES, H. R., PETERSON, W. H., and FRED, E. B. A rapid method for the determination of sugar in bacterial cultures. Jour. Bact. **12**: 427–439. 1926.

16. THOR, C. J. B., and SMITH, C. L. A physiological study of seasonal changes in the composition of the pecan during the fruit development. Jour. Agr. Res. **50**: 97–121. 1935.

STIMULATIVE EFFECTS OF X–RAYS ON PLANTS

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(WITH ELEVEN FIGURES)

Introduction

The majority of previous investigations dealing with the effects of x-rays on plants have been concerned with the question of growth stimulation of the aerial portion of the plant resulting from treatments with weak doses of x-rays (**4, 5, 6, 8, 9, 13, 14**). The methods generally employed involved an x-ray treatment of unsoaked or soaked seeds, or of growing points. Observations recorded during subsequent growth, particularly at maturity, were examined for evidences of stimulation. Reports of the stimulative effects of x-rays on the growth of the aerial portions of the plants are numerous but in only a few cases is the evidence conclusive. Inadequate sampling and limited test periods have been given as reasons for regarding the evidence as inconclusive.

The view that a consistent growth stimulation effect might be obtained by using a specific x-ray dose, was emphasized by SHULL and MITCHELL (**21**). They employed metallic screens, high voltage, low amperage, and short exposures. The use of metallic screens eliminated the soft x-rays.

In addition to the x-ray treatment, however, it is also necessary to consider the condition of the test material. Chemical alterations, generally destruction, and re-synthesis are commonly affected by x-ray irradiation and, in biological materials, these may well be the fundamental factors concerned. Such x-ray-induced changes would undoubtedly alter plant growth in view of its chemical nature owing to the presence and activities of auxins, calines, growth inhibitors, and other growth factors (**18**). The experiments reported in this investigation were not designed to determine this chemical aspect of growth stimulation; it is worthy of consideration, however, in future experiments of this type.

It is the purpose of the work described here to investigate the question of stimulation of primary root growth in seedlings of seeds given a small dose of soft x-rays. Previous work of RIVERA (**16, 17**) reported stimulative effects of treating roots with small doses of x-rays. This, however, was not a study on root growth stimulation directly, but rather on the resulting growth acceleration of aerial portions of plants following the irradiation of the roots.

In experiments described in this paper, corn was found to be most suitable for the method of germination employed. Stimulation as it will be used throughout refers to an increase in root length, or a greater wet or dry weight of roots of seedlings from treated seeds as compared with that occurring in the roots of normal seedlings. Observations were limited to the first five days of germination and the data presented refer solely to that period.

Methods

The x-ray radiation was provided by a gas type x-ray tube (12) having a copper target, operated at 10 ma. and at various voltages mentioned later. The seeds which were irradiated were arranged with embryos toward the source of radiation at a distance of eight cm. from the focal spot of the tube. The window of the tube, which was made of aluminum foil and Cellophane, transmitted the K_a and K_β characteristic radiation (1.54 and 1.38 Å.) as well as that of both longer and shorter wavelength radiation of the continuous spectrum of the copper target. The shorter wavelength emitted in
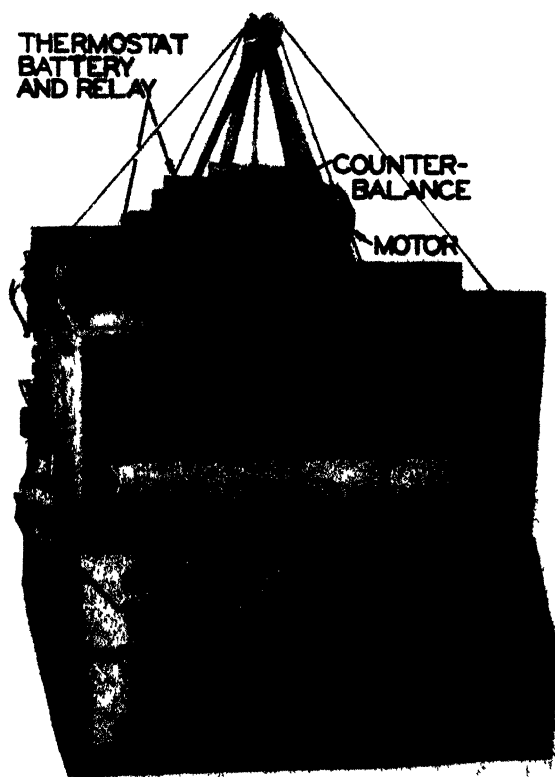
Fig. 1. Apparatus for seedling germination under controlled experimental conditions.

any experiment was approximately 0.5 Å. In every case, the time of exposure was 10 seconds.

After considerable preliminary work for the purpose of determining the requirements necessary to obtain sufficient data, it was decided to construct an apparatus which would permit 2000 seeds to be germinated simultaneously in water culture under controlled conditions. The apparatus (fig. 1) consisted of a large wooden cabinet in the bottom of which was a tank containing tap water kept at 29° C. by thermostatic control. A uniform

temperature of the water was maintained by the use of a stirring motor equipped with paddles to circulate the water in the tank. A constant flow of fresh water through the tank was made possible by small entrance and exit tubes.

The frame which supported the seeds during germination consisted of a rack suspended from above by four cables. The position of the rack below or above the water level was automatically controlled by means of a crank operated by an electric motor. Across the rack were placed 40 metal rods, each equipped with 50 fine nickel wire hooks. The purpose of each hook was to support a corn seed in its normal position by passing the hook through the endosperm portion of the seed at a point removed from the embryo.

The corn used in the experiments was Stowell's Evergreen Sweet Corn, 1940 crop. Badly formed and inferior kernels were discarded. The selected seeds were prepared for germination, the first part of which involved a twenty-four-hour soaking period, as follows: Fifty corn seeds were placed in each of forty copper wire baskets designed as seed-soaking containers. Twenty of these baskets of seeds were arbitrarily chosen as controls and the seeds in each of the remaining twenty baskets were given identical x-ray treatments.

It was found by trial that fifty roots, that is the content of one rod, could be examined for length and wet weight in a ten-minute interval. Consequently, in order to have all plants of the same age when observations on root growth were made, the following procedure was adopted: Fifty seeds were irradiated, replaced in a particular basket and the basket suspended from the rod to which the seeds were to be attached after soaking. Ten minutes later a basket containing non-irradiated seeds was suspended from the next rod. After another ten-minute interval a basket of irradiated seeds was suspended from the third rod. This procedure of starting the alternate soaking of control and irradiated seeds every ten minutes was continued for each of the 40 rods.

After a 24-hour soaking period the seeds were attached to the hooks of the rod which had suspended the basket. When the seeds were arranged on the wire hooks, a mechanism was started which automatically controlled the movement of the entire rack to positions below or above the water level. Preliminary experimentation indicated that good germination was possible if the germinating seeds were immersed in water for five-minute periods followed by two-hour intervals during which time they were suspended above the water.

At the end of the germinating period of four days, the rods were removed from the apparatus at ten-minute intervals in the same order as originally arranged, so that the age of the seedlings from the time that the seeds were first put to soak until they were harvested, was exactly five days. As each rod with its lot of seedlings was taken from the rack, the roots of the plants were severed as close to the seed as possible. The length of each root was measured and the total wet weight of the roots from the seedlings on this

rod was determined. The roots were then placed on paper and dried in the open air in the laboratory for seven days, after which time the total dry weight of the roots was determined. A seedling which did not produce root growth of at least two mm. in length was considered as not germinating.

Results

The results obtained show such a degree of variability that it is difficult to make a reliable interpretation of them without the use of statistical analysis. Tests of significance (7) were applied in comparing the experimental values obtained from the irradiated seeds with those obtained from the non-irradiated controls to determine whether the differences in these values could be ascribed, without much doubt, to a difference in the treatments received by the samples or whether they could be due to random sampling.

A comparison of the results obtained has been made with regard to the following variates:

The number of seeds germinating on each rod, x_1.

The total root length per rod, x_2.

The average root length per seed germinating on each rod, x_3.

The length of the longest root on each rod, x_4.

The total length of the 25 longest roots on each rod, x_5.

The total wet weight of roots per rod, x_6.

The average wet weight of root per seed germinating on each rod, x_7.

The total dry weight of roots per rod, x_8.

The average dry weight of root per seed germinating on each rod, x_9.

Each of the nine varieties was tested in two ways. First, the control seeds and irradiated seeds were regarded as separate and distinct samples chosen at random from the same population group. The difference between the mean value of a variate for the controls and for the irradiated seeds was tested to see if this difference was significant. Second, each rod of irradiated seeds was paired with an adjacent rod of control seeds. The differences of the variates for each pair was tested to see if its mean value differed significantly from zero. The first test led to a probability value p_1, which measured the probability of choosing from the same population group two random samples having mean values which differed by at least as much as that observed. The second test led to a probability value p_2 which measured the probability of choosing from a population group having a zero mean value a random sample having a mean value which differed from zero by as much or more than that observed. The usual convention of selecting a probability level of 5 per cent. or less as being significant has been adopted.

The percentage stimulation based upon the mean value of each variate for the control and for the irradiated seeds is given in Table I. The parentheses enclose those values for which one or both tests for significant dif-

TABLE I

PERCENTAGE STIMULATION OF VARIATE. BASED ON MEAN VALUE OF VARIATE

EXPERIMENT NUMBER	PEAK KILOVOLTS	r UNITS PER MINUTE	X_1 NUMBER OF SEEDS GERMINATING	X_2 TOTAL ROOT LENGTH PER ROD	X_3 AVERAGE ROOT LENGTH PER SEED GERMINATING ON EACH ROD	X_4 LENGTH OF LONGEST ROOT ON EACH ROD	X_5 TOTAL LENGTH OF 25 LONGEST ROOTS ON EACH ROD	X_6 TOTAL WET WEIGHT OF ROOTS PER ROD	X_7 AVERAGE WET WEIGHT OF ROOT PER SEED GERMINATING ON EACH ROD	X_8 TOTAL DRY WEIGHT OF ROOTS PER ROD	X_9 AVERAGE DRY WEIGHT OF ROOT PER SEED GERMINATING PER ROD
			%	%	%	%	%	%	%	%	%
1	20.0	1000	(6.2)*	(10.5)	(4.2)	3.0	(5.4)	(10.1)	3.7	(10.2)	4.0
2	15.0	450	-1.1	2.6	3.9	2.8	2.3	2.5	3.2	2.6	3.3
3	0.0	0	-17.0	1.4	3.4	4.2	2.1	1.2	3.3	2.2	4.3
4	17.5	750	-0.1	2.6	2.2	1.2	2.7	(8.6)	(8.3)	4.3	4.3
5	0.0	0	-2.2	-1.3	0.1	-0.5	-1.8	3.2	(5.2)	0.6	2.6
6	22.5	1300	0.1	-0.8	-0.3	-1.1	-1.0	-3.1	-2.7	-0.6	-1.0
7	20.0	1000	(7.2)	(10.2)	2.2	1.4	(3.6)	(8.8)	1.2	(9.2)	1.4
8	25.0	1700	0.4	-1.6	-2.2	0.7	-1.0	-5.6	-5.7	-0.8	-1.6
9	20.0	1000	-3.0	0.4	(3.3)	-1.5	1.0	0.6	3.7	-0.8	(3.3)

* Numbers in parentheses are those values for which one or both the tests for significance of differences indicated a real difference in the treatments of the controls and the irradiated seeds.

TABLE II
MOST PROBABLE LENGTH OF ROOT (15-MM. INTERVAL)

EXPERI-MENT NUMBER	PEAK KILO-VOLTS	r UNITS PER MINUTE	MIDPOINT OF INTERVAL		FREQUENCY		PERCENTAGE STIMULA-TION
			C	R	C	R	%
1	20.0	1000	101.0	112	172	183	10.9
2	15.0	450	94.0	102	154	148	8.2
3	0.0	0	86.0	89	104	221	3.5
4	17.5	750	75.0	86	185	202	14.7
5	0.0	0	71.0	74	171	180	4.2
6	22.5	1300	81.0	76	212	218	− 6.2
7	20.0	1000	86.0	105	173	193	22.1
8	25.0	1700	130.0	127	159	133	− 2.3
9	20.0	1000	119.5	127	216	201	6.3

ference indicated a real difference in the treatments of the controls and irradiated seeds. Except in one case, the only significant differences were obtained from samples irradiated at 17.5 to 20 peak kv. This one exception was in experiment 5 for which the voltage was zero. Since there was no difference in the treatments of those seeds marked "rayed" and those marked "control," this difference must be attributed to chance.

Using a 15-mm. class interval for the frequency tables of the root lengths of seedlings, the modal class for the seedlings from the control and irradiated seeds are given in table II. The percentage increases in the midpoint of this interval for the seedlings from the irradiated seeds over that for the control seedlings are given in this table. The averages of the percentage increases at zero kv. and 20 kv. are 3.8 and 13.1, respectively. The tests of significance do not indicate that the averages differ significantly from zero or from each other. The average of the increases at 17.5 and 20 peak kv. is 13.5 per cent. This value does differ significantly from zero but not from the results obtained at zero kv.

The percentages of stimulation at the various voltages are represented graphically in figures 2 to 11.
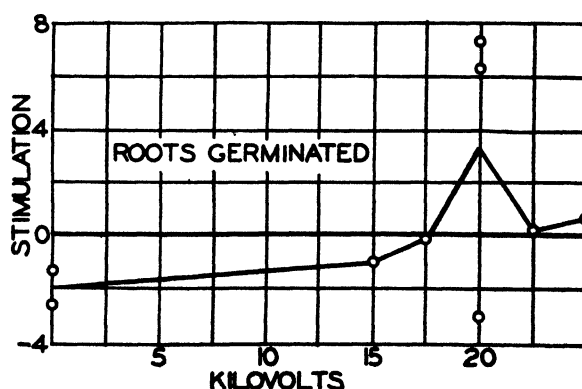
FIG. 2. Percentage stimulation determined from the number of seeds germinating on each rod (x₁) as a function of voltage.
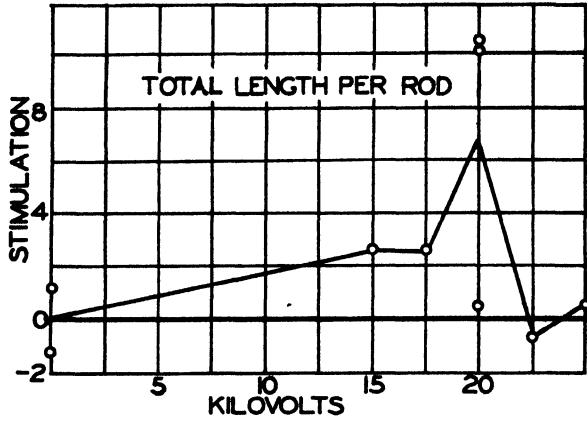
FIG. 3.   Percentage stimulation determined from the total root length per rod (x_2) as a function of voltage.
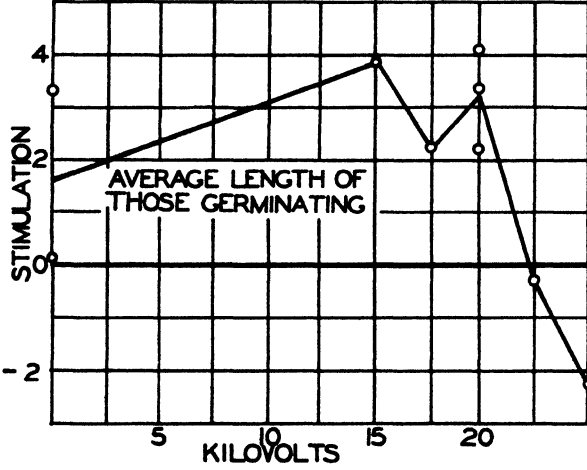
FIG. 4.   Percentage stimulation determined from the average root length per seed germinating on each rod (x_3) as a function of voltage.
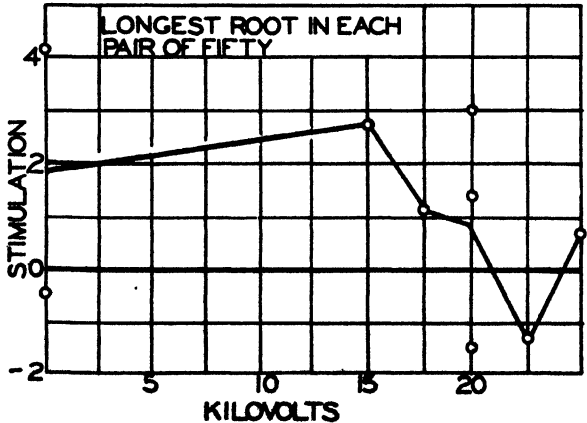
FIG. 5.   Percentage stimulation determined from the length of the longest root on each rod (x_4) as a function of voltage.
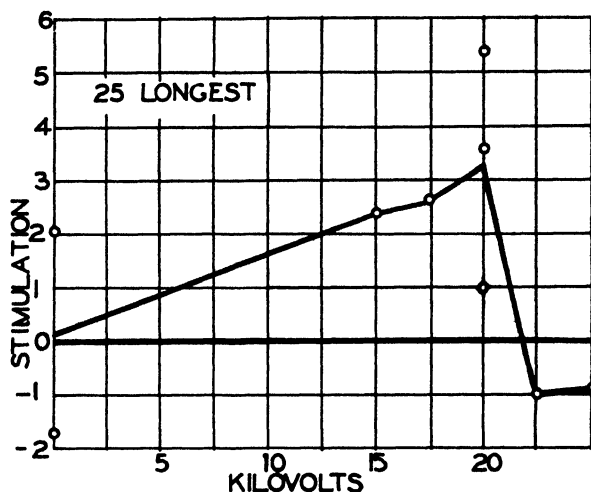
FIG. 6. Percentage stimulation determined from the total length on the 25 longest roots on each rod (x) as a function of voltage.
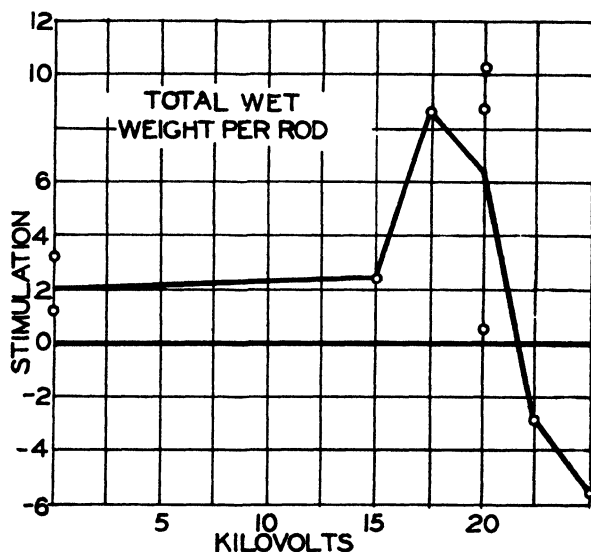
FIG. 7. Percentage stimulation determined from the total wet weight of roots per rod (x_u) as a function of voltage.

Discussion

No very great stimulation in primary root growth of corn seedlings from seeds given weak x-ray treatments as compared with that of control seedlings was obtained. Statistical analysis of the data, however, indicates beyond a reasonable doubt that the treatment of these seeds by x-rays employing a voltage in the neighborhood of from 17.5 to 20 peak kv. did produce a significant increase in the average length and in the wet and dry weights of the roots of the seedlings. Voltages of 22.5 and 25 peak kv. did not produce a significant change in any of the varieties examined.

PLANT PHYSIOLOGY
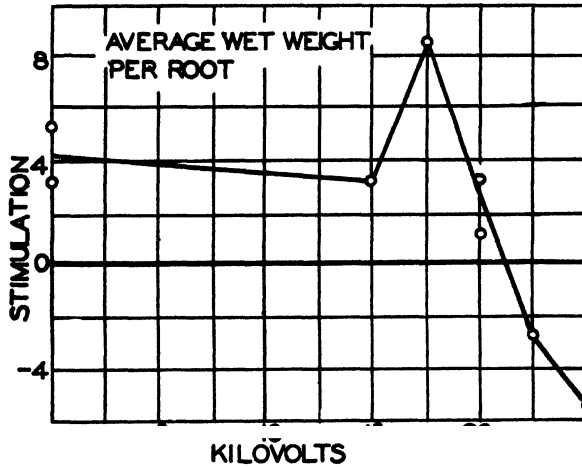
FIG. 8. Percentage stimulation determined from the average wet weight of root per seed germinating on each rod (x_7) as a function of voltage.
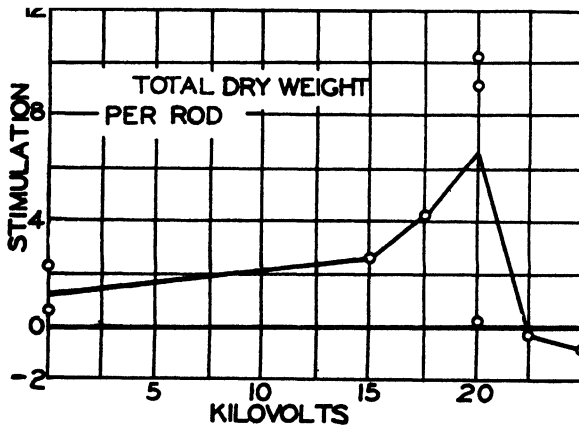
FIG. 9. Percentage stimulation determined from the total dry weight of roots per rod (x_8) as a function of voltage.
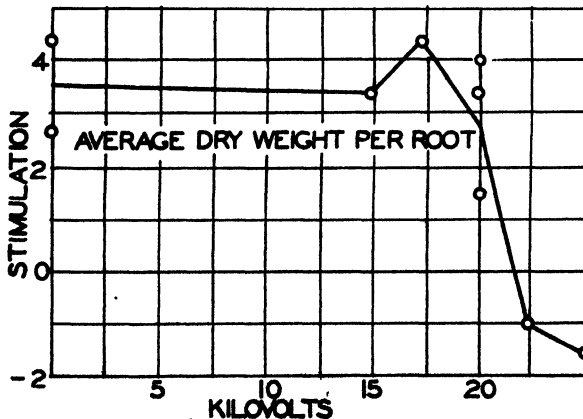
FIG. 10. Percentage stimulation determined from the average dry weight of root per seed germinating on each rod (x_9) as a function of voltage.

It is suggested by the data that the amount of radiation as well as the condition of the biological material to be treated which might induce growth stimulation may be very sharply defined. A slight change from the optimum condition for growth stimulation may fail to produce sufficient stimulation to detect or may even induce growth inhibition.
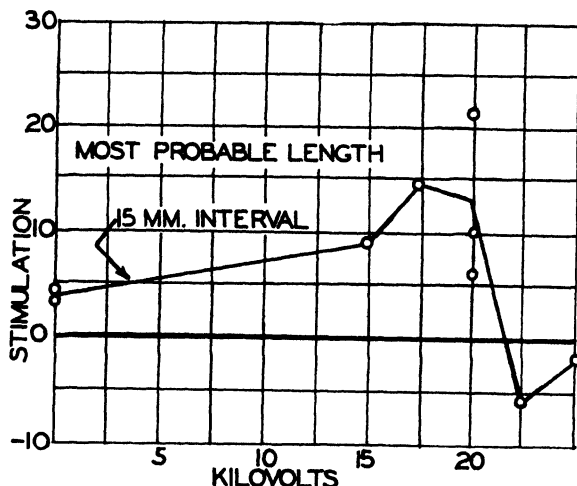
FIG. 11. Most probable root length (15 mm. intervals) as a function of voltage.

Summary

1. An apparatus for seedling germination which fulfills requirements of adequate sampling necessary to obtain reliable conclusions in investigations of x-ray induced growth stimulation has been described.

2. Under the conditions of these experiments it is possible to obtain an apparent x-ray-induced stimulation in the primary root growth of *Zea mays* seedlings. Statistical analysis of the data obtained indicates beyond a reasonable doubt that stimulation of root growth was obtained by irradiating dry seeds at voltages in the neighborhood of 17.5 to 20 peak kv. The analysis shows that differences as much as those observed would occur in fewer than five cases in one hundred trials if the x-ray treatment made no essential difference in root growth.

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LITERATURE CITED

1. ANCEL, SUZANNE. Action de faibles doses de rayons X sur des grains sèches. Compt. Rend. Soc. Biol. (Paris) **91**: 1435–1436. 1924.

2. ——————. Compt. Rend. Soc. Biol. (Paris) **96**: 986–989. 1927.

3. ——————. De l'influence acceleratrice des rayons X sur le developpement des plantes. Arch. Phys. Biol. **5**: 105–118. 1926.

4. BENEDICT, HARRIS M., and KERSTEN, H. Effect of soft x-rays on the germination of wheat seeds. Plant Physiol. **9**: 173–178. 1934.

5. BERSA, E. Strahlenbiologische Untersuchungen. I. Zur Frage der Roentgenreizwirkung bei Keimlengen. Sitzungsber. d. Acad. Wiss. Wien. Math. Naturw. Klasse. Abt. I. **135**: 424–456. 1926.

6. DUGGAR, B. M. Biological effects of radiation, Vol. II. McGraw-Hill. 1936.

7. GOULDEN, C. H. Methods of Statistical Analysis. Pp. 40–42. John Wiley & Sons. 1939.

8. JOHNSON, EDNA A. Growth and germination of sunflowers as influenced by X-rays. Amer. Jour. Bot. **15**: 65–76. 1928.

9. —————. The effects of x-rays upon growth and reproduction of tomato. Plant Physiol. **14**: 685–694. 1931.

10. —————. The alleged stimulating action of x-rays upon plants. Amer. Jour. Bot. **18**: 603–614. 1931.

11. —————. Growth of wheat plants from dry and soaked x-irradiated grains. Plant Physiol. **14**: 493–504. 1939.

12. KARSTEN, H. A gas x-ray tube for irradiation with soft x-rays. Radiology **23**: 60–63. 1934.

13. KOERNICKE, M. Wirkung von Roentgenstrahlen auf die Keimung und das Wachstum. Ber. d. bot. Ges. **22**: 148–155. 1904.

14. —————. Ueber die Wirkung verschieden starker Roentgenstrahlen auf Keimung und Wachstum bei den hoeheren Pflanzen. Jahrb. Wiss. Bot. **56**: 416–430. 1915.

15. MIEGE, E., and COUPE, H. De l'influence des rayons X sur la vegetation. Compt. Rend. Acad. Sci. (Paris) **159**: 338–340. 1914.

16. RIVERA, VINCENZO. Azione die raggi Rontgen sopra meristemi normali di Ricinus communis. Boll. R. Staz. Pat. Veg. **6**: 144–152. 1926.

17. —————. Introduzione allo studio della radiosensibilita die *Vicia faba*. Riv. Biol. **8**: 505–539. 1926.

18. SKOOG, FOLKE. The effect of x-irradiation on auxin and plant growth. Jour. Cellular and Comp. Physiol. **7**: 227–270. 1935.

19. —————. The effect of x-rays on growth substance and plant growth. Science n.s. **79**: 256. 1934.

20. SMITH, G. F., and KERSTEN, H. Root modifications induced in *Vicia faba* by irradiating dry seeds with soft x-rays. Plant Physiol. **16**: 159–171. 1941.

21. SHULL, C. A., and MITCHELL, J. W. Stimulative effects of x-rays on plant growth. Plant Physiol. **7**: 287–296. 1933.

EFFECTS OF DROUGHT, TEMPERATURE AND NITROGEN ON TURF GRASSES[1]

J. C. CARROLL

(WITH ONE FIGURE)

Introduction

The factors of major importance in the distribution of grasses are moisture and temperature, and observations have shown that management plays an important part in the duration of grasses. The present investigation was initiated to determine: (a) the relative survival of a number of turf grasses under different degrees, or intensities, of moisture and temperature, and (b) the effect of nitrogenous fertilization on these responses.

NEWTON and MARTIN (21) in a study of drought resistance of some crop plants in southern Alberta, including a number of grasses, found that bound water content could be used as a basis of classifying many of these plants in order of their drought resistance. On the other hand WHITMAN (30), working with four representative grassland types in western North Dakota, was unable to correlate bound water content with the ability of the species to withstand drought. VASSILIEV (27), VASSILIEV and VASSILIEV (28) believed that sugars play an important rôle in the drought resistance of wheat. TUMANOV (26) showed that repeated wilting of sunflowers resulted in increased hardiness to drought, and similar results with wheat plants were obtained by AAMODT and JOHNSTON (2).

BRIGGS and SHANTZ (5) in their review of previous work, state that many investigations have shown the water requirement of plants to be reduced by the use of fertilizers, the reduction being marked on poor soils, but slight on fertile soils. In a search for methods to eliminate quack grass, DEXTER (10) found that heavy fertilization with nitrogen lowered its resistance to drought. DEXTER (9) and CARROLL and WELTON (7) found that high-nitrogen plants failed to harden to cold as much as low-nitrogen plants.

MAXIMOV (15) in his investigations came to the conclusion that the true measure of drought resistance is the ability of the plant to survive exposure to drought without permanent injury. NEWTON (19, 20) found that he could distinguish cold-hardy from non-cold-hardy varieties of wheat on the basis of their bound water content. He also observed the higher sugar content of the hardy varieties as compared with that of the non-hardy, but assigned no particular significance to it. On the other hand, CHANDLER (8), and later ÅKERMAN (3), MEYER (17), and MAXIMOV (15), stressed the idea that soluble carbohydrates may increase frost resistance of plants by decreasing the precipitation of proteins.

Among recent investigators (15, 18) it appears to be the consensus of

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opinion that drought and cold resistance cannot be accurately predicted by any of the physico-chemical methods yet devised, and that for the most reliable results the method of direct testing should be employed. Equipment used in making drought studies has been described by SHIRLEY (**24**), AAMODT (**1**), and BAYLES, TAYLOR, and BARTEL (**4**), and use of artificial refrigeration in cold studies has won general acceptance.

Materials

The grasses included in the present investigations were: Kentucky bluegrass (*Poa pratensis* L.), Canada bluegrass (*P. compressa* L.), roughstalked meadow grass (*P. trivialis* L.), wood meadow grass (*P. nemoralis* L.), annual bluegrass (*P. annua* L.), Astoria bent and Cocoos bent (*Agrostis tenuis* L.), South German mixed bent (*A. tenuis* L., *A. palustris* L., and *A. canina* L.), Highland velvet bent (*A. canina* L.), red fescue (*Festuca rubra* L.), Chewings fescue (*F. rubra fallax* L.), sweet vernal (*Anthoxanthum odoratum* L.), perennial rye (*Lolium perenne* L.), Italian rye (*L. multiflorum* L.), crested dogstail (*Cynosurus cristatus* L.), redtop (*Agrostis alba* L.), and large crabgrass (*Syntherisma sanguinale* (L.) Dulac). Although desirable, the seed of the species used was not genetically pure; and it is recognized that tests similar to those reported in this paper should be made of the many strains within each species. The plots 4 by 50 feet were first seeded April 26, 1938, and reseeded September 6, 1939. The soil was a Wooster silt loam and in a high state of productivity. The results of the soil drought and high temperature tests reported here were obtained from the later seeding, while those of the low temperature and physico-chemical tests were made on the grass of both seedings. Owing to weather conditions at Wooster during the summer and early fall of 1940 neither soil nor air temperatures were extreme. Thus the turf samples subjected to soil drought and high temperatures were not hardened naturally at the time of sampling. The samples exposed to soil drought may have hardened while drying.

The plats were maintained under lawn conditions and were divided into two equal sections as follows: (1) Low nitrogen— no treatment: and (2) High nitrogen—ammonium sulphate applied at rate of 5 lb. per 1000 sq. ft. in April, July, and September.

GENERAL METHODS

A golf-green cup-hole cutter was used to cut samples of sod from the plats. By means of this tool "plugs" of sod 4¼ inches in diameter and approximately 3 inches in thickness were lifted from each plat and placed in glazed earthenware jars of the same dimensions. Before the samples were transferred to the jars, the plugs were "sized" with a knife so that the surface of the soil was approximately one-fourth inch below the edge of the jar. As a result of this procedure an approximate uniformity of soil mass among the samples was obtained, and they could be watered easily at any time. The grasses were clipped either with a lawn mower just previous

to sampling, or with shears afterward. The plugs of sod were subsequently exposed to different degrees of drought and temperature under experimental conditions. The dry weight of each plug was estimated by sampling the adjacent soil for moisture determinations. These estimates proved to be reliable when tested by the dry weights of the plugs at the conclusion of several of the treatments. Occasional tests of moisture in the plants were also made during the period of the investigation.

In the preliminary tests, the percentage of survival was obtained by counting the number of living and dead plants at the end of 3 weeks after each test was completed. Each stem was considered as a separate plant. It was perceived that such a method of evaluating results would be too time-consuming when a full complement of samples was taken. Therefore, in order to shorten the time required to evaluate results, the percentage of survival on a limited number of samples was estimated first by the writer and then one other person. These estimations were followed by actual counts. The difference between the percentages of survival obtained by count and by estimation was not significant, and the latter method was used in the evaluation of results reported in this paper.

Soil drought

The relative survival of the different grasses when exposed to soil drought was tested by permitting the soil to dry slowly and exposing the samples to an air temperature of 35° C. for 4 hours daily until the moisture of the soil had reached 5 per cent. in one series and 3 per cent. in a second series, on a dry-weight basis. These values are below the wilting coefficient of Wooster silt loam, which has been found to be about 7.7 per cent. The time of drying varied from 7 to 8 days for the 5 per cent. series and was approximately 2 days longer for the 3 per cent. series. The moisture content of the soil was ascertained by weighing daily until the end point was nearly reached, then weighing more frequently. To insure uniform drying below the wilting point the plugs were raised and supported to allow circulation of air on all sides.

These conditions of soil moisture and air temperatures were chosen because they are similar to those occasionally encountered in the field in this section of the United States. During periods of drought at Wooster in the summers of 1931 and 1936, the moisture in the first 4 inches of soil beneath Kentucky bluegrass sod became as low as 3.2 per cent.

In late summer, drying took place in the greenhouse, where the air temperature was maintained at approximately 35° C. for 4 hours. In the fall, when the greenhouse temperatures were generally below 25° C., the samples were placed in a Freas draft oven at 35° C. for 4 hours late in the day. By this procedure the later samples were not exposed to a materially shorter day than were the samples tested earlier in the season. There was no essential difference between results obtained by greenhouse and oven heating of samples. LAUDE (12) has shown that in plants previously exposed to light, the heat resistance is lost very gradually.

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After the percentage of water in the soil of the two series has been re-
duced to 5 per cent. and 3 per cent., respectively, sufficient water was added
to bring the moisture content to approximately 70 per cent. of the water-
holding capacity of the soil, and at the end of 3 weeks, an estimation was
made of the percentage of recovery. This percentage of .recovery is consid-
ered an index of the relative drought resistance of the different species of
grasses studied (table I).

The results of the tests on soil drought at the two percentages of mois-
ture content show that at 5 per cent. there was little or no injury among
the grasses from the low-nitrogen section with the exception of *Cynosurus
cristatus* (table I). The majority of the species from the high-nitrogen

TABLE I

SURVIVAL OF CERTAIN TURF GRASSES AS AFFECTED BY THE NITROGEN AND WATER
CONTENT OF THE SOIL

GRASS	PERCENTAGE OF SURVIVAL*			
	5 PER CENT. SOIL MOISTURE		3 PER CENT. SOIL MOISTURE	
	LOW-N SECTION	HIGH-N SECTION	LOW-N SECTION	HIGH-N SECTION
	%	%	%	%
Poa pratensis	100	70	80	50
Festuca rubra fallax	100	90	75	35
Agrostis tenuis (Cocoos)	100	40	75	40
A. tenuis (Astoria)	95	70	70	45
A. canina (Highland)	100	50	70	40
Festuca rubra	90	80	65	45
So. German mixed bent	80	80	60	40
Syntherisma sanguinale	80		60	
Agrostis alba	90	90	55	50
Poa compressa	80	55	50	35
P. annua	85	70	50	35
Lolium perenne			50	
Anthoxanthum odoratum	90	80	45	30
Poa trivialis	80	80	40	35
P. nemoralis	80	60	35	15
Cynosurus cristatus	60	30	20	20

* Average of estimations in 6 tests.

section, however, were definitely injured. At 3 per cent. moisture, marked
differences among the species from both the low- and high-nitrogen sections
were obtained (table I). Since approximately 2 days more were needed
to reduce the soil moisture to 3 per cent. than to 5 per cent., it is highly
probable that the time factor may be of more significance than the decrease
of soil moisture from 5 to 3 per cent., for both these percentages are below
the wilting coefficient of Wooster silt loam.

The effect of soil drought was tested six different times, and the results
were, in general, very consistent. Among all the species tested, *P. pratensis*
was consistently the most resistant, and *C. cristatus* was the least resistant
to soil drought. As might be expected, there was considerable variation

among the species of each genus.   In the Poa genus, *P. pratensis* far out-ranked others of the group included in this test on soil drought.   On the basis of past observations on our own turf plats, as well as from reports in the literature, *P. trivialis, P. nemoralis,* and *P. annua* were not expected to withstand a high degree of soil drought, and this expectation was amply ful-filled in the present tests.   *P. compressa* has been reported as being more drought resistant than *P. pratensis* (**22**) but in the tests made during this study, it was a very poor second, ranking only slightly above *P. trivialis,* which is notoriously non-hardy under drought conditions.

Different reasons have been assigned in the literature as to why either *P. pratensis* or *P. compressa* dominates any given locality to the virtual ex-clusion of the other, but there seems to be little agreement as to causes. BROWN (**6**) in testing four pasture grasses, including *P. pratensis* and *P. compressa,* under controlled temperature conditions, found that when both air and soil temperatures were maintained at 100° F. for approximately 2 months, *P. compressa* was injured much more than *P. pratensis.*   He con-cluded that the destructive effects resulted more from a high soil tempera-ture than from a high air temperature.

During the periods in which the writer's samples were exposed to an air temperature of 35° C., the soil temperature eventually equaled the air temperature.   The highest soil temperature attained in this test was lower than that in the test by BROWN (**6**), and maintained for a considerably shorter period, but the relative amount of injury in the two grasses was similar.   In the present experiments, therefore, it appeared that the effect was due chiefly to soil drought, and not to high soil temperature.

The species of the genera Agrostis and Festuca which were tested did not differ materially among themselves, and, as can be seen from table I, there was little difference between these two groups and *P. pratensis,* with the exception of South German mixed bent and *A. alba,* which were injured much more than the other grasses in the bent group.

The relatively high survival of the fescues was expected, for experience and observation had shown them to be fairly resistant to drought.   On the lawn plats at Wooster, one plat of Chewing's fescue, located on the south side of a maple tree, has maintained a good stand since being seeded in 1928, with no attention other than being mowed at regular intervals.   In that span of years, there have occurred several periods of extreme drought, but the stand of Chewing's fescue has not been seriously affected.   This species is a valuable ground cover in locations where low moisture conditions exist, as on terraces.   In contrast, *P. trivialis,* located on an adjacent plat, has had to be reseeded annually, since it has died out each season within the space of a few weeks after the inception of hot, dry weather.   In a study of the water-supplying power of soil under different grasses, WELTON and WILSON (**29**) found that fescues apparently absorb less of the available soil moisture than do bluegrass and bents, and WILSON and LIVINGSTON (**31**) in a study of the wilting of grasses as related to the water-supplying power of

the soil found the fescues to be more drought-resistant than the other species tested.

Of the other grasses included in this study, *A. alba* and *L. perenne* are the most important from the standpoint of their value in pastures and in production of turf. The fact that they rank low in survival when exposed to soil drought may partially account for their gradual elimination by other grasses generally included in turf mixtures.

The effect of liberal applications of nitrogen on the survival of the different grasses on dry soil is shown in table I. In all cases, the recovery of the grasses from the high-nitrogen section was less than that from the low-nitrogen section, and in most cases the difference was marked (fig. 1). In a few cases only, was there a significant difference in the percentage of survival among the grasses from the high-nitrogen section.

EFFECT OF HARDENING ON RESISTANCE TO SOIL DROUGHT

To determine the effect of repeated wilting on the drought hardiness of grasses, duplicate samples of five species representing four genera were taken on one of the dates when all the species were sampled for the usual tests. The same procedure was followed as in the regular tests, with the exception that when the soil was dried to approximately 5 per cent. moisture, sufficient water was added to bring it back to the original moisture condition, after which it was left to dry to 3 per cent.

The results (table II) show that with *P. pratensis* and *F. rubra fallax*, there was no increase in drought hardiness, but that in *P. compressa* and *C. cristatus*, an increase in hardiness was obtained by one previous drying. *P. pratensis* and *F. rubra fallax* probably harden more quickly than the other grasses tested.

High soil and air temperatures

It has been found by numerous workers that large fluctuations in soil temperature are confined chiefly to the upper layers, also that on bare soil in summer, the temperature of the upper soil layer is generally greater than

FIG. 1. Effect of soil drought on low- and high nitrogen grasses at 3 per cent. soil moisture content.

JAR NO.	GRASS	TREATMENT
1	*P. pratensis*	Low N
2		High N
3	*P. compressa*	Low N
36		High N
9	*P. annua*	Low N
10		High N
11	*A. tenuis* (Astoria)	Low N
12		High N
17	*A. canina* (Highland)	Low N
60		High N
23	*Anthoxanthum odoratum*	Low N
24		High N
27	*A. alba*	Low N
28		High N
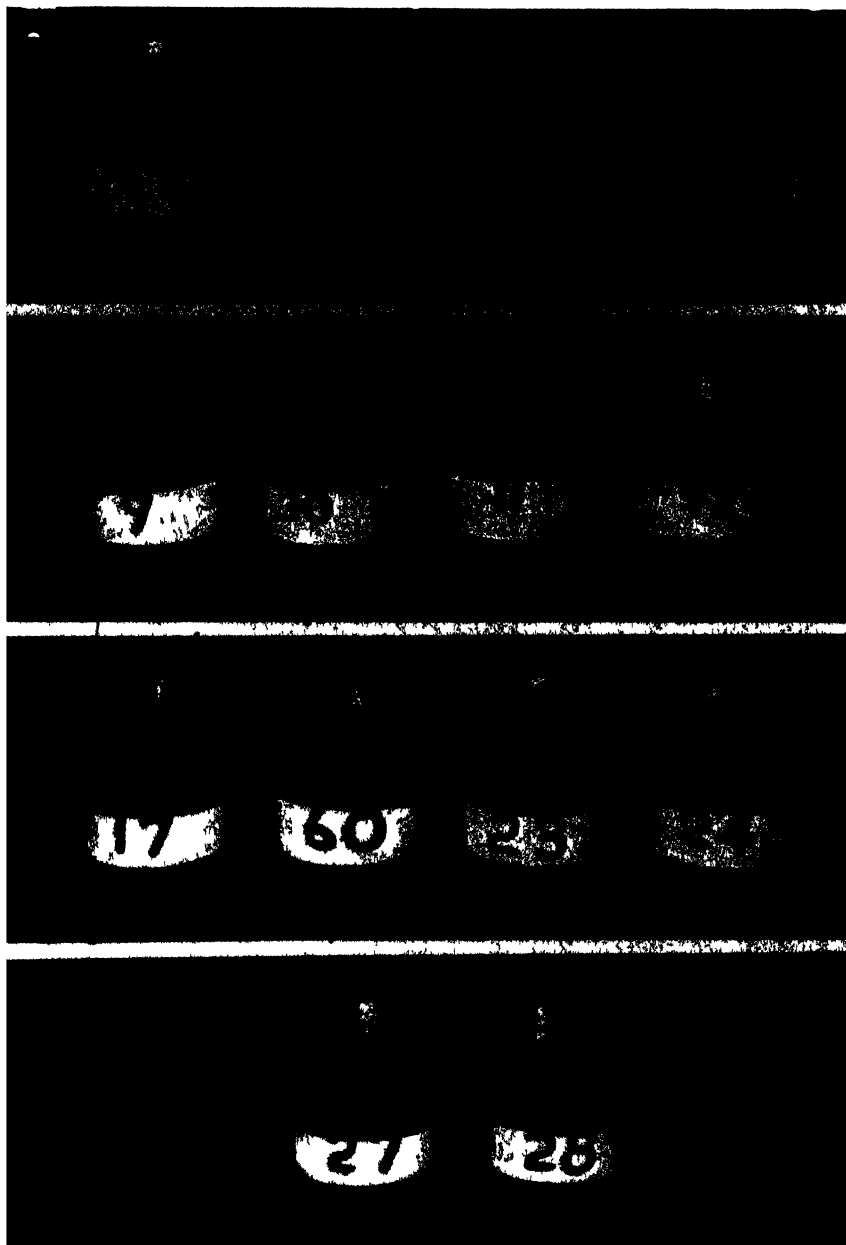
TABLE II

COMPARATIVE EFFECTS OF REPEATED DRYING VERSUS SINGLE DRYING ON SOIL DROUGHT
ENDURANCE OF CERTAIN TURF GRASSES

GRASS	PERCENTAGE OF SURVIVAL AFTER DRYING TO 3 PER CENT.	
	SINGLE DRYING	REPEATED DRYING*
	%	%
P. pratensis	80	80
P. compressa	50	80
A. tenuis (Astoria)	70	50
F. rubra fallax	75	75
C. cristatus	20	60

* Soil dried to approximately 5 per cent. moisture, watered, and then dried to 3 per cent.

that of the air. SMITH (25) reported temperatures of 146° F. and 107° F.,
respectively, at ½-inch and 3-inch depths in bare soil with an air temperature
of 105° F. Reports in the literature on soil temperature beneath sod are
very fragmentary. FITTON and BROOKS (11), in a survey of soil tempera-
ture studies, list only a few readings taken beneath sod. The maximum
temperature during August under a bluegrass sod at a 2-inch depth was
73.2° F.; the air temperature was not given. In an orchard sod at a depth
of 3 inches, the temperature was 76.4° F. with an air temperature of 70.2°
F.; the kind of grass was not named.

Records of air temperature at different heights above the soil surface are
more numerous and more complete than those of soil temperature. These
include measurements taken above different types of cover. Temperatures
of 105° F. to 115° F. have frequently been reported in the central section
of the United States. ROBB (23) at the Topeka, Kansas, Weather Bureau
has reported air temperatures as high as 116° F. at a height of 5 feet above
sod.

Owing to weather conditions at Wooster during the summer season of
1940, neither soil nor air temperatures were extreme. The summer season
was above average in rainfall and below average in temperature, with only
about 2 weeks of hot, dry weather which occurred during the latter half
of July. Soil temperatures at a depth of 2 inches on the turf plats rose
during that period to a maximum of 86° F. at 2:00 P.M., July 22, with an air
temperature of 91° F.

The temperature of the soil beneath the grasses on the high-nitrogen sec-
tion was invariably lower than that on the low-nitrogen section, due, no
doubt, to the higher moisture content of the soil in the high-nitrogen section.

HIGH SOIL TEMPERATURE

To determine the effect on turf grasses of high soil temperature accom-
panied by high soil moisture, 60 jars of sod representing 4 replications of
each grass were placed on a tray in a steel tank containing water heated by

means of a steam coil.   The soil temperature was measured by a thermometer inserted to a depth of 1½ inches in the center of each plug of sod.   The water was circulated while being heated in order to maintain an even temperature throughout the tank.   Within about 2 hours, the temperature of the soil became the same as that of the surrounding water.   In one test, the soil temperature was held at 50° C. for 4 hours; in the other test, the jars were removed as soon as the maximum temperature was attained.   The results of subjecting the grasses to a high soil temperature are recorded in table III.

Soil temperatures frequently influence plant survival more than air temperatures.   Since the high soil temperature in these experiments was not accompanied by a high air temperature, the initial injury in this case was limited to the underground portions of the plant.   As the moisture content of the soil was comparatively high, the injury could not have been due to desiccation.   The experiment was not continued long enough to bring about a condition of starvation through increased respiration.   It appears, therefore, that the injury was due to thermal effects upon the protoplasm.   From data available in the literature, it appears that most non-dormant plant cells are heat-killed by temperatures of 50° C. to 60° C., sometimes even lower.   The lethal temperature is lower with a slow rise in temperature than with a comparatively more rapid rise (**13**).

Merely bringing the soil temperature to 50° C. was not lethal to the grasses growing in the low-nitrogen section, but most of these same species in the high-nitrogen section were injured by this treatment (table III).   Where the grasses were held at 50° C. for 4 hours, the injury was much greater

TABLE III

SURVIVAL OF CERTAIN TURF GRASSES AS AFFECTED BY THE NITROGEN CONTENT AND HIGH TEMPERATURES OF THE SOIL

GRASS	PERCENTAGE OF SURVIVAL					
	50° C.*		60° C.*		4 HR. AT 50° C.	
	LOW N SECTION	HIGH N SECTION	LOW-N SECTION	HIGH-N SECTION	LOW N SECTION	HIGH N SECTION
	%	%	%	%	%	%
Syntherisma sanguinale					85	
A. canina (Highland)	100	100	20	2	70	15
F. rubra fallax	100	90	70	0	65	10
P. trivialis	100	90	5	5	60	20
P. annua	100	70	20	0	60	55
F. rubra	100	90	25	0	45	7
P. compressa	100	100	70	50	40	20
A. tenuis (Cocoos)	100	70	1	0	40	7
A. alba	100	80	0	0	40	5
P. pratensis	100	100	2	2	25	40
Anthoxanthum odoratum	100	60	10	0	20	1
P. nemoralis	100	65	2	0	15	1
So. German bent	100	70	1	1	15	5
Cynosurus cristatus	100	100	0	0	15	5
A. tenuis (Astoria)	100	50	0	2	3	2

* Jars removed as soon as maximum soil temperature was attained.

(table III).   Thus, the lethal temperature varies with the time of exposure.
In all three experiments, the time necessary to bring the soil temperature
to the maximum was approximately 2 hours.   From the data in table III,
it is evident that the underground portions of a number of these grasses
cannot long endure soil temperatures between 50° and 60° C.   The greater
injury occurred in the grasses from the high-nitrogen section.

Results indicate that *P. compressa* is better able to withstand high soil
temperatures than *P. pratensis*.   This result is in contrast to the results
obtained by BROWN (6), who in a study of the effect of combined high soil
and air temperatures on the growth of the two grasses, found that *P. com-
pressa* was injured more than *P. pratensis*.   He concluded that the effect
was due to high soil temperature rather than to high air temperature.   In
his tests, however, the grasses were kept at a soil and air temperature of
100° F. for approximately 8 weeks, and a starvation effect due to a high rate
of respiration was probably obtained.   In the test reported here, the tem-
perature of 50° C. (122° F.) for 4 hours probably produced a more direct
killing effect.   Owing to its habit of growth, *P. compressa* shades the soil
less than *P. pratensis,* and one would expect higher daily soil temperatures
on some of the sites where *P. compressa* dominates.   Its relatively more
frequent dominance on shallow soils above rocks adjacent to deeper soil
areas dominated by *P. pratensis* is probably related to its endurance of
short periods of high soil temperatures and low fertility rather than of
drought.

A. canina (variety Highland), which was found to be fairly resistant to
soil drought, was the most resistant to high soil temperature (50° C. for 4
hours) of any of the grasses tested, and *F. rubra fallax* ranked second.   The
other bent grasses were much less resistant to high soil temperatures than
was Highland; but tests later in the fall showed that they survived low tem-
peratures much better than Highland.   It appears, therefore, that the
changes in the inner conditions of plants which enable them to endure
extremes in temperature are not necessarily the same for both heat and cold.
This difference is further borne out in a limited way by the results of mea-
surements of bound water which show no significant difference between
grasses representing extremes in drought, heat, and cold endurance (table
VII).   These data further substantiate the argument of those who have
declared the inadequacy, at the present time, of physico-chemical analyses
as reliable indices of the relative resistance of plants to extreme conditions
of temperature and moisture.

HIGH AIR TEMPERATURES

The effect of short exposures to high air temperatures on turf grasses
was investigated by placing the grasses for different intervals of time in a
Freas draft oven.   The temperatures used were 40°, 50°, and 60° C., and
the time, 4 and 6 hours at each temperature.   The exposure to heat was made
in the evening, as in the experiments on soil drought.   In this test, however,

the moisture content of the soil remained relatively high, but the relative humidity of the air passing over the samples was appreciably lower (approximately 15 per cent. at 50° C.) than obtained in the test on soil drought. Four replicates of each grass were tested.

In this experiment, as in the test on high soil temperatures, the results were doubtless due to thermal effects. However, in exposing the grasses to air temperatures similar to those used in the high soil temperature tests, much less injury was obtained. The reason for this cannot be stated definitely. With some grasses, the extent and type of root development may be the explanation; with others, the presence of rhizomes enabling the plant to send up new shoots when the original aerial portions have been destroyed may be of greater importance. Again, as has been suggested by Maximov

TABLE IV

SURVIVAL OF CERTAIN TURF GRASSES WHEN EXPOSED TO AN AIR TEMPERATURE OF
50° C. FOR 6 HOURS

GRASS	PERCENTAGE OF SURVIVAL	
	LOW N SECTION	HIGH-N SECTION
	%	%
P. pratensis	80	40
A. tenuis (Cocoos)	70	20
A. canina (Highland)	70	55
A. tenuis (Astoria)	65	15
P. trivialis	60	35
P. annua	60	40
Agrostis alba	60	20
P. compressa	50	2
So. German mixed bent	50	25
Syntherisma sanguinale	45	
Anthoxanthum odoratum	40	2
F. rubra fallax	20	5
F. rubra	20	7
P. nemoralis	10	5
Cynosurus cristatus	7	2
Final soil temperature	40.1° C.	

(16), the ability of the stomatal mechanism to withstand high temperatures may play an important part in the heat resistance of a plant.

The effects on the grasses of heating at 40° C. for 4 and 6 hours were negligible; there was only a slight injury to the tips of some leaves on both low- and high-nitrogen sections. In the test at 50° C. for 4 hours, there was very little injury to any of the grasses on the low-nitrogen section, but there was much greater injury to the grasses on the high-nitrogen section. Of all the exposures, the greatest difference among the grasses was obtained at 50° C. for 6 hours (table IV). The species of Poa and Agrostis used in this investigation, with the exception of *P. nemoralis* and South German mixed bent, withstood high air temperatures better than the species of the other genera tested. With few exceptions, those grasses having the greater resistance to soil drought also endured high air temperature better than

those with a lower resistance to soil drought. The exceptions particularly noted were the fescues, which were much less resistant to high air temperature than to soil drought. These results are at variance with those observed under outdoor conditions, but it is extremely unlikely that the low relative humidity prevailing in this test and the limited amount of soil would be duplicated under natural conditions.

LOW TEMPERATURE

The extent of injury to a plant due to cold depends upon the type of plant and upon the nature of its preconditioning. Most perennial plants

TABLE V

PERCENTAGE OF SURVIVAL OF TURF GRASSES FOLLOWING A SINGLE COOLING TO DIFFERENT SOIL TEMPERATURES

Grass	PERCENTAGE OF SURVIVAL WITH A FINAL SOIL TEMPERATURE OF							
	− 5° C.		− 10° C.		− 15° C.		− 20° C.	
	Low-N	High N	Low N	High-N	Low N	High-N	Low-N	High-N
P. pratensis	100	90	80	60	25	5	20	5
P. compressa	90	80	30	30	5	2		
P. trivialis	90	50	45	25	0	0	0	0
P. nemoralis	80	30	70	5	0	1		
P. annua	80	50	40	10	3	1	5	0
A. tenuis (Astoria)	90	80	60	50	0	0	0	0
A. tenuis (Cocoos)	90	75	80	45	1	0	0	0
So. German mixed bent	90	75	70	50	0	0	0	0
A. canina (Highland)	100	20	5	5	0	0		
A. alba	90	90	60	40	0	0	0	0
F. rubra fallax	90	65	75	30	0	0	0	0
F. rubra	80	65	60	25	0	0	0	0
Anthoxanthum odoratum	50	30	20	10	0	0	0	0
Cynosurus cristatus	40	20	0	0	0	0	0	0
L. perenne	70	20	0	0	0	0	0	0
L. multiflorum	40	10	10	0	0	0	0	0

growing in the temperate region can, to some extent, be hardened to cold and are thus enabled to withstand lower temperatures than when not hardened. In the present study, a comparison of the endurance of different grasses to cold was made on samples from both the unfertilized and the fertilized sections of the plats. The grasses were permitted to harden naturally. Previous work (7) had shown that air temperatures near the freezing point were necessary to harden turf grasses. That nitrogen treatments may affect the degree of hardening of turf grasses to cold was shown by CARROLL and WELTON (7).

The technique employed to test the survival of species of turf grasses

exposed to low temperature by artificial refrigeration was essentially the same as that described previously (7). Samples from the low- and high-nitrogen sections of each grass were placed in the freezing chamber at −25° C., and as soil temperatures of −5°, −10°, −15°, and −20° C., were reached, duplicate samples of each grass for each treatment were removed. The samples were then placed in the greenhouse and after 3 weeks, an estimation was made of the percentage of survival.

The results obtained by exposing the grasses to cold until definite soil temperatures were attained are shown in table V and represent 6 replications. Only three grasses from the low-nitrogen section were appreciably injured at a soil temperature of −5° C., but injury of the high-nitrogen grasses was more general. This difference between grasses from the low- and high-nitrogen sections held throughout the temperature range of the test, except where the injury of a particular species was complete in both sections. *P. pratensis* was found to be the most resistant to low temperatures of any of the grasses tested. However, within the range of low soil temperatures generally encountered in the field at Wooster, *F. rubra fallax* and some of the Agrostis species survived nearly as well as *P. pratensis*.

Soil temperatures beneath sod at Wooster during the winter of 1940–1941 were not low enough to injure any of these turf grasses except the least cold-resistant ones on the high-nitrogen sections. At a depth of 1½ inches, the soil temperature was not lower than −2° C., as the ground was covered by a thin covering of snow during the coldest periods. MAIL (14), in studying the effect of soil temperatures on insect survival, found that under a light covering of snow (¾ inch to 2 inches), the soil temperature remained within 1° or 2° of the freezing point, whereas on sod-covered ground, without snow cover, a soil temperature of −10° C. was recorded at a depth of 2 inches, when the air temperature was −13° C.

Since the degree of hardening of turf grasses to cold was decreased on the sections to which high amounts of nitrogen had been applied, it would appear inadvisable to supply grass with any considerable amount of this fertilizer constituent late in the fall. The time of application should, of course, be determined by the prevailing weather conditions, so that a luxuriant growth late in the season would be avoided. BROWN (6) found that Kentucky and Canada bluegrass and orchard grass made considerable growth at 40° F. In a study of the comparative effect of different carriers of nitrogen on growth of Kentucky bluegrass, WELTON and CARROLL (unpublished data) found little diminution of the weekly absorption of nitrates in late fall when the daily maximum and minimum air temperatures were, respectively, slightly above and below 40° F.

EFFECT OF SOIL DRYING ON HARDENING TO COLD

Statements have appeared in the literature (26) to the effect that decreasing the moisture supply of plants facilitates their hardening to cold. To test whether this statement might be true of turf grasses, two lots of five

species were hardened to cold; one lot with an optimum soil moisture, and the other with a soil moisture slightly above the wilting percentage. After hardening by daily exposure to a temperature of approximately 0° C. for 1 week, the grasses were placed in a freezing chamber and removed as soon as the soil temperature had been lowered to – 10° C. The time necessary to cool the soil to this minimum temperature varied from approximately 3½ hours for the samples with high moisture content to 1½ hours for those with the low moisture content. The results (table VI) show that drying of the

TABLE VI

COMPARATIVE EFFECTS OF HARDENING BY COLD ONLY VERSUS HARDENING BY COLD PLUS SOIL DRYING ON THE COLD-ENDURANCE OF CERTAIN TURF GRASSES

GRASS	PERCENTAGE OF SURVIVAL AFTER FREEZING TO – 10° C.*		
	UNHARDENED	HARDENED BY COLD	HARDENED BY COLD PLUS SOIL DRYING (SINGLE DRYING)
	%	%	%
P. pratensis	0	80	75
P. compressa	0	30	45
A. tenuis (Astoria)	0	60	15
F. rubra fallax	0	75	25
C. cristatus	0	0	10

* – 10° C. soil temperature.

soil resulted in no significant gain in cold hardiness by three of the grasses and was detrimental to the remaining two. On the latter, the greater cold injury may have been due to the more rapid lowering of the soil temperature, and not to the extremity of the minimum temperature itself.

Physico-chemical tests

Numerous attempts have been made to discover physico-chemical measurements by which it would be possible to predict the cold or drought resistance of plants. Measurements of bound water and sugars have perhaps received the greatest attention. The evidence obtained has been conflicting.

Clippings from a number of the most and the least cold- and drought-hardy species of turf grasses were taken at several different dates and analyzed for moisture, bound water, total nitrogen, and total sugars (table VII). From previous tests (7) it was known that with the approach of cold weather, there was an elaboration of hydrophilic colloids in *P. pratensis* and an increase in the total sugar content, accompanied by increased hardiness to cold, but at that time no comparison was made among different species.

In tests made in August and October, there was a lack of correlation between the bound water content of the different species and their demonstrated survival, or lack of survival, when exposed to drought. This disparity is particularly noticeable when *F. rubra fallax* is contrasted with *C. cristatus*, *P. trivialis* and *P. nemoralis*. Within the same species, the high-

TABLE VII

DATA FROM PHYSICO CHEMICAL ANALYSES OF CERTAIN TURF GRASSES

GRAMS PER 100 GRAMS OF FRESH TISSUE

GRASS	SECTION	AUGUST Total Moisture	AUGUST Bound Water	OCTOBER Total Moisture	OCTOBER Bound Water	NOVEMBER Total Moisture	NOVEMBER Bound Water	DECEMBER Total Moisture	DECEMBER Bound Water	OCTOBER Total Sugars	NOVEMBER Percentage of nitrogen in dried tissue
		gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	%
P. pratensis	Low N	72.4	7.42	71.6	7.92	73.5	9.68	64.1	9.56	5.40	2.52
	High N	76.2	7.04	75.3	7.64	72.3	7.28	67.0	7.36	4.97	3.94
P. trivialis	Low N	73.8	6.32	75.4	6.72	77.3	6.38	63.0	7.48	4.42	2.98
	High N	77.0	6.48	81.0	5.86	79.1	5.26	76.4	5.74	4.01	3.86
P. nemoralis	Low N	73.2	7.26	75.3	7.58	75.8	7.42	66.0	9.96	4.15	2.44
	High N	75.8		76.8	6.86	79.1	6.16	68.6	7.02	3.74	3.72
A. tenuis (Astoria)	Low N	71.8	7.12	73.8	7.71	71.9	9.18	67.5	9.16	5.17	2.70
	High N	76.6	6.98	76.0	6.41	74.3	7.04	71.2	7.84	4.17	4.23
A. canina (Highland)	Low N	72.7	6.86	73.0	7.13	72.4	8.76	66.8	9.43	4.73	2.61
	High N	76.2		75.9	6.54	75.7	7.00	70.9	8.13	4.06	4.03
F. rubra fallax	Low N	69.8	6.38	74.4	6.82	77.3	7.30	66.9	9.46	4.12	2.07
	High N	74.2		76.5	5.08	79.3	6.36	67.3	7.63	3.81	3.70
C. cristatus	Low N	71.3	6.48	75.0	7.08	72.0	7.90	62.6	9.36	4.53	2.16
	High N	75.8	6.62	80.4	5.14	82.0	5.82	64.2	7.18	3.92	3.44

nitrogen grass generally contained less bound water than the low-nitrogen grass and was always less resistant to drought.   It should be noted that weather conditions previous to these tests were not conducive to hardening to drought.   In tests conducted on *P. pratensis* in 1930 and 1931, however, when weather conditions favorable to hardening to drought prevailed, CARROLL and WELTON (7) found a lack of correlation between bound water content and drought hardiness in low- and high-nitrogen grass.

In November and December tests, which were made after the grasses had hardened to cold, there was a greater increase of bound water content in the low-nitrogen grasses than in the high-nitrogen grasses, and the low-nitrogen grasses possessed greater hardiness to cold.   The differences in bound water content among a number of the grasses from the low-nitrogen section were negligible, but there were wide differences among them with respect to cold hardiness.   Here, again, it must be said that on the basis of these tests, bound water cannot be accepted as a reliable index to the cold resistance of grasses.

Summary

A comparison was made of the survival of 15 species, representing 6 genera, of turf grasses exposed to soil drought and to extremes in soil and air temperatures.   The effect of nitrogenous fertilizers in conjunction with these conditions was also investigated.

The term, soil drought, as used in this report, refers to conditions in the soil as it slowly dried to a moisture content of 5 per cent. or of 3 per cent., which is from 2.7 per cent. to 4.7 per cent. below the moisture content found to be the wilting coefficient of the Wooster silt loam on which the grasses were grown.   Of the species tested, *P. trivialis, P. nemoralis,* and *C. cristatus* were most injured by soil drought.   Those least injured were *P. pratensis, F. rubra fallax, F. rubra, A. tenuis* (Astoria and Cocoos species), and *A. canina* (Highland).   The species from the high-nitrogen section were less able to withstand soil drought than the same species from the low-nitrogen section.

The species were exposed to an air temperature of − 25° C. until soil temperatures of − 5°, − 10°, − 15°, and − 20° C. were reached.   The species most injured by low soil temperatures were *L. perenne, L. multiflorum, C. cristatus, A. odoratum,* and *A. canina* (Highland).   Those least injured were *P. pratensis, P. nemoralis, A. tenuis* (Cocoos), South German mixed bent, and *F. rubra fallax.*   The lethal soil temperature for the majority of the species appeared to be between − 10° and − 15° C.   The species from the high-nitrogen section were much less resistant to cold than those from the low-nitrogen section.   Low soil temperature was more injurious than low air temperature.

Exposure to soil and air temperatures of 40°, 50°, and 60° C. showed that high soil temperatures were more destructive than similar air temperatures.   The injury caused by high temperature of both soil and air appeared to be a direct thermal effect upon the protoplasm.   The lethal temperature

varied with the time of exposure and for most species appeared to be between 50° and 60° C. Species from the high-nitrogen section suffered greater injury than those from the low-nitrogen section.

The data from tests made of bound water and sugars showed that the accumulation of these two constituents was decreased by the application of a nitrogenous fertilizer. These data were found to be unreliable as criteria of the relative hardiness of turf species to heat, cold, or drought.

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LITERATURE CITED

1. AAMODT, O. S. A machine for testing the resistance of plants to atmospheric drought. Canadian Jour. Res. **12**: 788–795. 1935.

2. ——————, and JOHNSTON, W. H. Studies on drought resistance in spring wheat. Canadian Jour. Res. **14**: 122–152. 1936.

3. AKERMAN, A. Studien über den Kältetod und die Kälteresistenz der Pflanzen nebst Untersuchungen über die Winterfestigkeit des Weizens. Lund. 1927.

4. BAYLES, B. B., TAYLOR, J. W., and BARTEL, A. T. Rate of water loss in wheat varieties and resistance to artificial drought. Jour. Amer. Soc. Agron. **29**: 40–52. 1937.

5. BRIGGS, L. J., and SHANTZ, H. L. The water requirement of plants. II. A review of the literature. U. S. Bur. Plant Ind. Bull. 285. 1913.

6. BROWN, E. MARION. Some effects of temperature on the growth and chemical composition of certain pasture grasses. Missouri Agr. Exp. Sta. Res. Bull. 299. 1939.

7. CARROLL, J. C., and WELTON, F. A. Effect of heavy and late applications of nitrogenous fertilizer on the cold resistance of Kentucky bluegrass. Plant Physiol. **14**: 297–308. 1938.

8. CHANDLER, W. H. The killing of plant tissue by low temperature. Missouri Agr. Exp. Sta. Res. Bull. **8**: 143–309. 1913.

9. DEXTER, S. T. The winter-hardiness of weeds. Jour. Amer. Soc. Agron. **29**: 512–517. 1937.

10. ——————. The drought resistance of quack grass under various degrees of fertilization with nitrogen. Jour. Amer. Soc. Agron. **29**: 568–577. 1937.

11. FITTON, EDITH, M., and BROOKS, CHARLES F. Soil temperatures in the United States. Mon. Weather Rev. **59**: 6–16. 1931.

12. LAUDE, H. H. Diurnal cycle of heat resistance in plants. Science n.s. **89**: 556–557. 1939.

13. LEPESCHKIN, W. W. Zur Kenntnis der Einwirkung supramaximaler

Temperaturem auf die Pflanze. Ber. d. bot. Ges. **30**: 703–714. 1912.

14. MAIL, G. ALLEN. Winter soil temperatures and their relations to subterranean insect survival. Jour. Agr. Res. **41**: 571–592. 1940.

15. MAXIMOV, N. A. Internal factors of frost and drought resistance in plants. Protoplasma **7**: 259–291. 1929.

16. ————. The plant in relation to water. London, Allen and Unwin. 1929.

17. MEYER, B. S. Seasonal variations in the physical and chemical properties of the leaves of the pitch pine, with especial reference to cold resistance. Amer. Jour. Bot. **15**: 449–472. 1928.

18. ————. Further studies on cold resistance in evergreens, with especial reference to the possible role of bound water. Bot. Gaz. **94**: 297–321. 1932.

19. NEWTON, R. A comparative study of winter wheat varieties with especial reference to winter killing. Jour. Agr. Sci. **12**: 1–19. 1922.

20. ————. Colloidal properties of winter wheat plants in relation to frost resistance. Jour. Agr. Sci. **14**: 178–191. 1924.

21. ————, and MARTIN, W. M. Physico-chemical studies on the nature of drought resistance in crop plants. Canadian Jour. Res. **3**: 336–427. 1930.

22. PIPER, C. V. Forage plants and their culture. The Macmillan Co. Revised ed. 1924.

23. ROBB, A. D. Comparison of temperatures from roof and ground exposures at Topeka, Kans., 1935–36. Mon. Weather Rev. **65**: 388–392. 1937.

24. SHIRLEY, H. L. A method of studying drought resistance in plants. Science n.s. **79**: 14–16. 1934.

25. SMITH, ALFRED. Daily and seasonal air and soil temperatures at Davis, California. Hilgardia **4**: 77–112. 1930.

26. TUMANOV, I. I. Ungenügende Wasserversorgung und das Welken der Pflanzen als Mittel zur Erhöhung ihrer Dürreresistenz. Planta **3**: 391–480. 1927.

27. VASSILIEV, I. M. The effect of drought on the carbohydrates of wheat plants. Bull. Appl. Bot. and Pl. Breed. **27**: 47–69. 1931.

28. ————, and VASSILIEV, M. G. Changes in carbohydrate content of wheat plants during the process of hardening for drought resistance. Plant Physiol. **11**: 115–125. 1936.

29. WELTON, F. A., and WILSON, J. D. Water-supplying power of the soil under different species of grass and with different rates of water application. Plant Physiol. **6**: 485–493. 1931.

30. WHITMAN, WARREN C. Seasonal changes in bound water content of some prairie grasses. Bot. Gaz. **103**: 38–63. 1941.

31. WILSON, J. D., and LIVINGSTON, B. E. Wilting and withering of grasses in greenhouse cultures as related to water-supplying power of the soil. Plant Physiol. **7**: 1–34. 1932.

EFFECTS OF CERTAIN GROWTH REGULATING SUBSTANCES ON GROWTH CORRELATION IN LETTUCE SEEDLINGS

RICHARD B. STEPHENSON

(WITH NINE FIGURES)

The cultivation of excised plant parts in tissue culture has been used as one of the means of studying the effects of growth substances on the development of certain tissues. Wide use of this technique has been made in studying the growth requirements of excised roots (6, 7, 8, 10, 12, 13). Other embryonic tissues (5, 9, 11) and callous tissue (14) also have been cultured in this way. Full utilization of this technique, however, has not been made for the study of the effects exerted by the different organs of the plant on one another, as well as for the influences exerted by the different growth regulating substances upon the organ.

The present experiments were planned with the belief that something could be learned about the effects of growth regulating substances on the development of excised parts, and also about certain correlations within the plant by aseptically culturing various parts of the plant separately and in association with one another. Consequently, in many of the experiments four series of cultures were made: (1) whole plants, (2) excised roots, (3) excised shoots, and (4) excised roots together with excised shoots in the same flask.

Since the especial purpose of the investigation was to observe the effect of growth regulating substances on the early development of the plant, and to locate as nearly as possible the organs affected by these substances, no attempt was made to find an optimum basic medium for any one organ or for the plant as a whole. The medium used was considered satisfactory, since better growth resulted for the whole plant in the basic medium over the two week period the cultures were grown than in soil or sand culture.

Materials and methods

Seeds of the Grand Rapids variety of tip burn resistant lettuce were first soaked for two hours in a saturated solution of Semesan and were then passed through a 5 per cent. solution of bromine water and transferred to sterile, water-soaked paper in sterile Petri dishes. All operations were carried out in a transfer chamber previously washed with a 4 per cent. solution of formalin. The seeds were allowed to germinate for forty-eight hours before transfers were made to the culture solution. The roots were then ten to twenty mm. long, and the shoot, including the cotyledons, about the same length. For the series of excised roots, the terminal ten mm. were severed from the plant with a Bard-Parker scalpel and transferred to the culture solution on the tip of the scalpel. With a little practice this maneuver could be carried out successfully and the possibility of injury with forceps avoided. The scalpel was flamed with 95 per cent. alcohol before each operation, and

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the necks of the culture flasks also were flamed.   Contamination was infrequent.   Shoots excised about 2 mm. below the cotyledonary node, as well as whole plants, were similarly transferred at this stage of development.   A standard WHITE's formula of salts (12) with 2 per cent. sucrose was used in making up the solution to which the growth substances were added. Water twice distilled in Pyrex was used in making up all solutions, as well as for the final washing of the flasks.   Fifty ml. of solution were added to each 125-ml. Pyrex culture flask.

All four series (excised roots separately, excised shoots separately, excised roots and shoots in the same flask, and intact plants) were cultured in each of the following concentrations of any particular growth substance: 1.0, 0.1, and 0.01 mg. per liter of the culture solution.   Intact plants and excised roots were cultured also in a concentration of 10 mg. per liter, which in most cases caused great inhibition.   Ten cultures were made of each series in each concentration.   Measurements of the pH were made of each flask as a matter of routine after the tissues were removed.   The data presented in table I show that the more actively growing tissues had induced a more alkaline reaction.

TABLE I

THE INFLUENCE OF THE RATE OF GROWTH UPON THE pH OF THE NUTRIENT SOLUTION, WHEN PLANTS WERE GROWN IN THE SOLUTION CONTAINING 0.1 MILLIGRAM OF THIAMIN PER LITER

ROOT GROWTH	pH
cm.	
16.4	5.52
21.8	5.65
22.1	5.87
25.9	5.90
26.1	5.99
26.9	6.00
30.8	6.20

Two criteria of growth were used in all cases: the length of root attained, and the dry weight of both the roots and the shoot.   The comparison of the length and the weight serves as an index for the type of root growth (i.e., long thin, short thick, etc.) taking place.   The cultures were grown in daylight at room temperature ranging from 20° to 25° C. for a period of two weeks.

Results

The observations on these sets of experiments may be grouped into the following classes: (1) those which show the effects of leaving available to the plant, or of blocking off, the various possible paths for the transmission of correlative factors; and (2) those which show the effects of the different growth regulating substances tested.   The factors correlating growth in the young lettuce seedling might be divided into two groups: those which could travel only through the living tissue of the plant; and those which could also

diffuse through a culture medium. It was for this reason that the excised parts were cultured together in the same medium, as well as in separate flasks. If histograms are made of the five series considered in class 1 for not only the controls but for each of the growth substances tested as well, then a simultaneous comparison of the effects from separating the plant parts and from the physiological effects of the chemicals may be made.

CORRELATION

Each histogram shows the mean of the total root growth per plant and represents the mean of at least ten cultures. Figure 1 represents the corre-
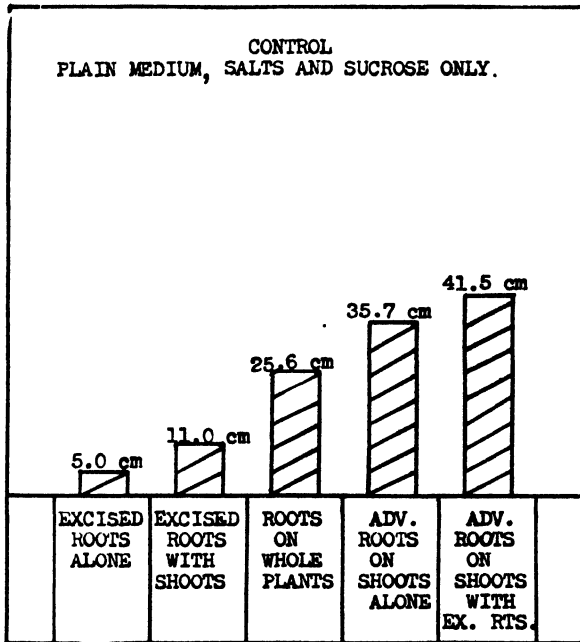
FIG. 1. Total mean root growth per plant in plain nutrient medium in the different correlation series. Excised root growth was slightly benefited by the presence of the shoot. Adventitious root growth on excised shoots exceeded total root growth on the intact plants. A further slight increase in adventitious root growth occurred when the excised root was present in the medium.

lation group occurring in the basic medium; i.e., salts and sucrose without added growth substance. The growth of the excised root alone, which is accountable only to those factors which were contained in the tip when it was excised, those which were manufactured by the root, and to the material of the culture medium, is the least of the five root types. The root which was cultured in the presence of the excised shoot, and which could be additionally influenced by substances diffusing from the growing shoot into the medium, grew slightly better. This effect, though slight, was very constant. The total root growth on the intact plants, including the main tap root, branch, and adventitious roots, is greater than that of any excised roots.

This growth could be influenced by factors produced in the shoot, and also by those in the growing tap root. That the latter is true is suggested by the growth of adventitious roots on excised shoots, which apparently are free from the restraining influence of some inhibitory substance associated with the main tap root. If the excised root is present in the same culture with the excised shoot, a further increase in adventitious root growth on the shoot occurs. This might indicate that the main tap root also supplies some substance stimulatory to root growth which may diffuse through the culture medium, and also that the inhibitory factor must pass through the living tissue of the plant. It is also possible that effects ascribed to the necessity
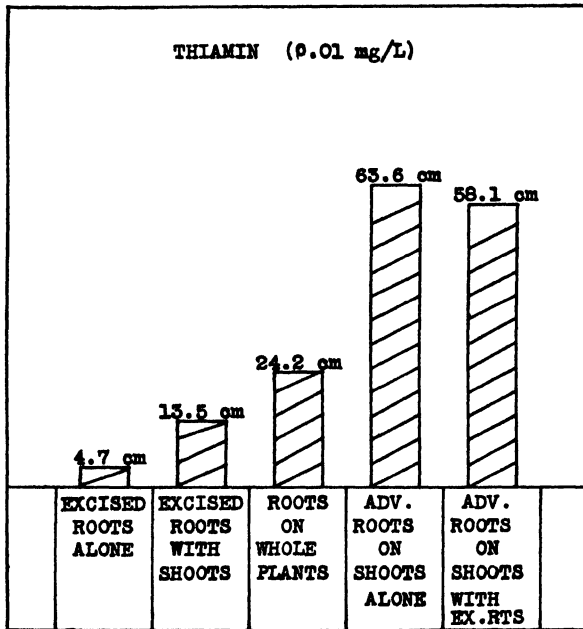
FIG. 2. Total mean root growth per plant with thiamin added to culture medium. The further stimulation of adventitious root growth on the excised shoots by the presence of the excised root as seen in the control series (fig. 1) did not take place.

for factors to pass through living tissue are actually due to the great dilution any substance must undergo while diffusing from any part of the plant through the culture medium.

Possible clues as to the nature of the factors, presumably "growth substances" of some sort, effecting these correlative differences, may be obtained from the histograms showing the growth that occurs when the different sets are grown in media to which the growth substances have been added. The substances used in these experiments were chosen at random from a large number of physiologically active substances which might have been used. No suggestion is made whatever that any of these actually take part in normal correlative activities within the plant, but it is pointed out that some of their effects are qualitatively similar to those obtained when the plant's normal paths of correlation are broken.

THIOUREA (0.01 mg/L)

46.7 cm   48.2 cm

25.4 cm

9.5 cm

5.1 cm

| EXCISED ROOTS ALONE | EXCISED ROOTS WITH SHOOTS | ROOTS ON WHOLE PLANTS | ADV. ROOTS ON SHOOTS ALONE | ADV. ROOTS ON SHOOTS WITH EX. RTS |
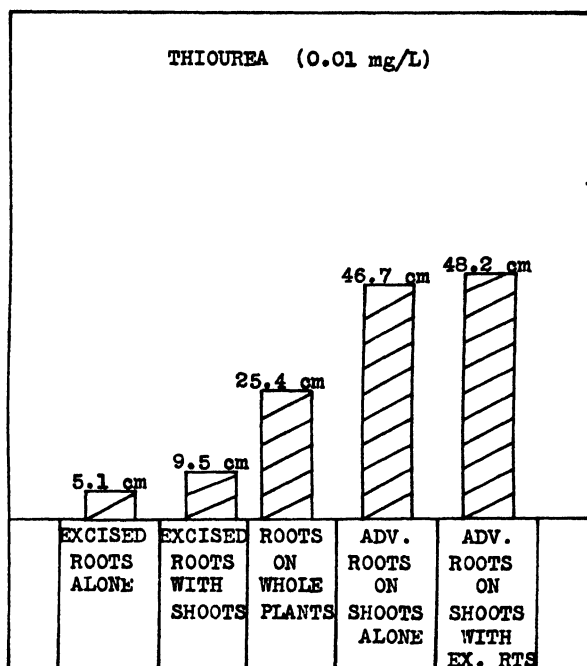
FIG. 3. Total mean root growth per plant with thiourea added to culture medium. This histogram does not appear to be significantly different from that for the control series (fig. 1).

NICOTINIC ACID (0.01 mg/L)

42.2 cm

32.9 cm   35.0 cm

6.3 cm   6.3 cm

| EXCISED ROOTS ALONE | EXCISED ROOTS WITH SHOOTS | ROOTS ON WHOLE PLANTS | ADV. ROOTS ON SHOOTS ALONE | ADV. ROOTS ON SHOOTS WITH EX.RTS |
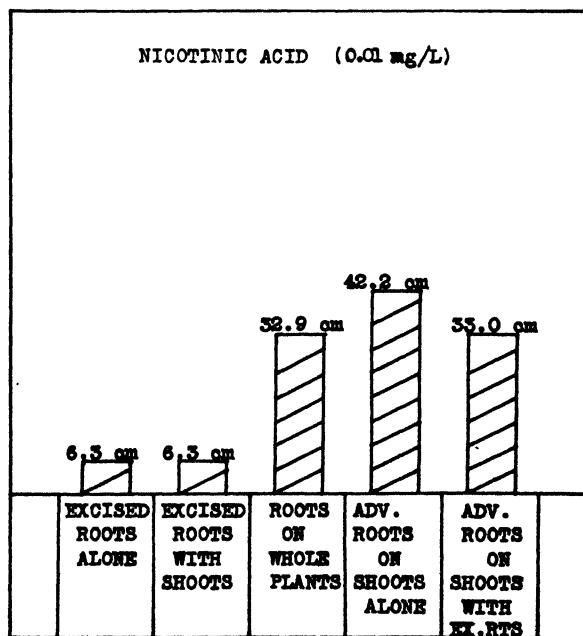
FIG. 4. Total mean root growth per plant with nicotinic acid added to culture medium. This figure is similar to that for thiamin (fig. 2) with the further difference from the control that there was no stimulation of the excised root by the shoot.

When thiourea (fig. 3) was added to the culture medium, the general trend of the histogram was not changed from that of the basic medium, and hence its rôle, if any, in correlation is not shown. Thiamin (fig. 2) does not greatly alter the shape of the figure, except that no increase in adventitious root growth on shoots cultured with excised roots occurs over that in those cultured alone. This is also true of nicotinic acid (fig. 4), in which, in addition, the characteristic slight increase in growth (for the control) of the excised root taking place in the presence of the shoot did not occur. Accordingly, it appears that the usual reciprocal relationship between the
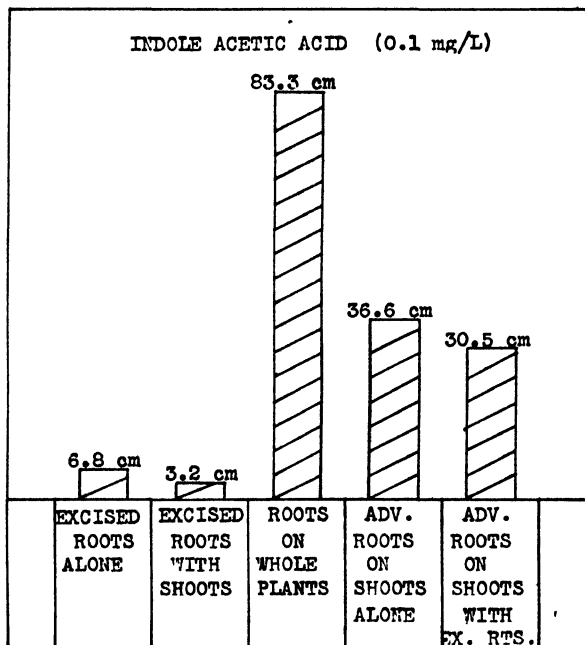
Fig. 5. Total mean root growth per plant with indole acetic acid added to culture medium. Here the growth of the excised roots alone was definitely greater than when in the presence of the shoot. The increased growth of roots on the intact seedling over shadowed the response of the adventitious roots both alone and in the presence of the root. These responses and relationships were all exactly the opposite of those observed in the control series.

root and the shoot in the control group is altered by thiamin and nicotinic acid.

The figures for indole acetic acid (fig. 5) and naphthalene acetic acid (fig. 6) are very similar, except for magnitude. The decreased growth of the excised root cultured with the shoot, as compared with that cultured alone, suggests that the substance diffusing from the shoot may be physiologically similar to these substances—that is, an auxin, since other experiments have shown that these will cause an increase in the growth of excised roots if the concentration is low enough, but are inhibitory in higher concentrations. The reversal in trend of the other parts of the figure may be due

in part to the large number of adventitious roots which are induced on the whole plant, and for which some interaction of substances conveyed through living tissues from both the main root and shoot seems to be necessary. This appears reasonable since the number of adventitious roots developing on excised shoots is less when auxins are present in the medium and also because those lateral roots which are induced on excised roots are very poorly organized. In high concentrations (ten milligrams per liter) of these substances. adventitious roots fail to develop at all.
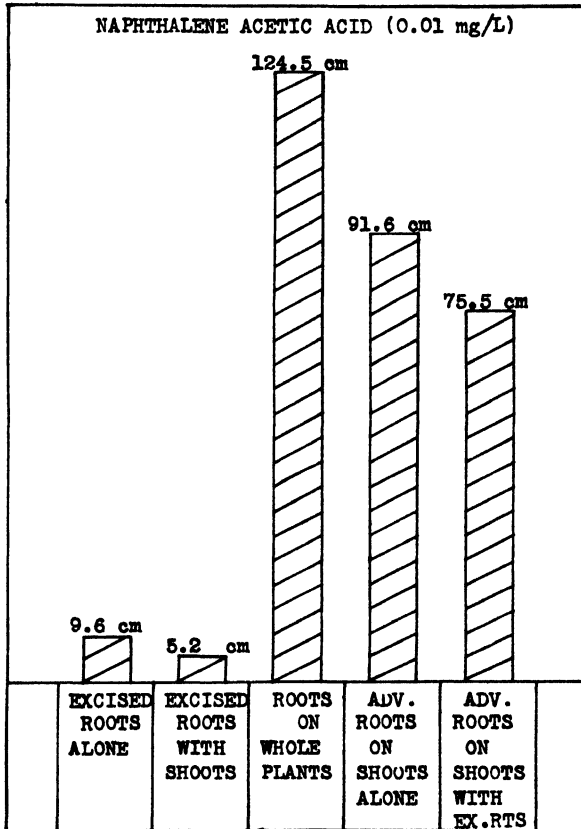
Fig. 6. Total mean root growth per plant with naphthalene acetic acid added to culture medium. The relationships seen here were like those in indole acetic acid, but with a wider latitude of response.

That naphthalene acetamide (fig. 7) also causes an increase in excised root growth, and that the growth of the root alone is greater than when it is with the shoot seems to indicate that in some cases the auxins with the free acid group may themselves be the acting agent in the plant. The rest of the group for naphthalene acetamide shows the trend typical of the basic medium rather than that typical of the auxins. This indicates that in some cases others of the diverse effects of these substances may occur through the formation of secondary compounds, such as the amide linkage.

GROWTH SUBSTANCES

The effects of the growth substances as compared with the controls are comprehensively shown in table II, which is complete for the two most extensively studied concentrations, 0.1 and 0.01 mg. per liter.

Many reports have been made of the effect of thiamin on plant growth. None of our findings indicate that it has a stimulatory effect on the growth of intact lettuce plants. This concurs with the results of BONNER and GREENE (3) with tomato and some other plants. These authors assume that most of the annual green plants are able to produce enough thiamin so that it does not limit root growth in the normal plant.
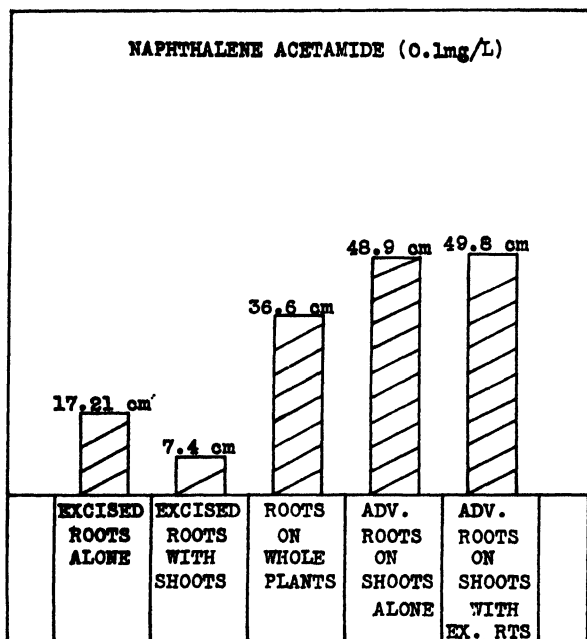
FIG. 7. Total mean root growth per plant with naphthalene acetamide added to culture medium. The excised roots showed a response similar to that found in naphthalene acetic acid and indole acetic acid, but root growth on the shoot and the intact plant was not greatly different from the control. This split response should be especially noted.

A concentration of ten mg. per liter of thiourea produced a slight increase in root growth and in the dry weight of the plant. This high concentration may be effective partly because of the mere added supply of nitrogen as a nutrient, but this does not seem probable. In other respects the results obtained with thiamin and thiourea were similar. In concentrations of 0.1 and 0.01 mg. per liter a consistent and comparable stimulation of adventitious root growth by both substances occurred on excised shoots. This was not always accompanied by an increase in dry weight, as these roots were very long, infrequently branched, and exceedingly small in diameter. It should be noted that the adventitious roots appear on the shoots in most cases within 24 hours after their transfer to the culture flask.

TABLE II

SHOWING THE EFFECTS OF THE VARIOUS SUBSTANCES ON GROWTH IN THE DIFFERENT SERIES. MEASUREMENTS EXPRESSED AS PERCENTAGE OF CONTROL MEAN FOR THAT SERIES

SUBSTANCE	CONCENTRATION PER LITER	EXCISED ROOTS ALONE		EXCISED ROOTS WITH SHOOTS		WHOLE PLANTS			SHOOTS ALONE			SHOOTS WITH ROOTS		
		LENGTH	DRY WEIGHT	ROOT LENGTH	DRY WEIGHT	ROOT LENGTH	DRY WEIGHT OF ROOT	DRY WEIGHT OF SHOOT	ROOT LENGTH	DRY WEIGHT OF ROOT	DRY WEIGHT OF SHOOT	ROOT LENGTH	DRY WEIGHT OF ROOT	DRY WEIGHT OF SHOOT
	mg.	%	%	%	%	%	%	%	%	%	%	%	%	%
Thiamin	0.10	119.6	94.7	130.5	176.2	88.6	74.4	105.0	160.7	170.3	102.1	135.7	105.6	115.2
	0.01	94.0	76.3	123.2	154.0	94.4	71.6	81.0	178.3	220.0	162.7	140.0	152.9	136.9
Thiourea	0.10	117.6	89.5	114.2	116.0	84.0	65.2	81.0	178.9	271.7	180.2	117.8	97.1	82.3
	0.01	101.0	26.7	86.7	128.6	98.9	100.4	74.8	131.0	106.7	88.4	116.0	110.0	110.1
Nicotinic acid	0.10	159.2	81.6	159.7	196.8	99.5	91.2	62.6	104.4	80.0	55.2	139.7	141.6	121.5
	0.01	125.0	139.0	57.8	71.4	128.4	147.0	102.7	118.3	95.0	70.8	79.5	85.7	79.3
Naphthalene acetamide	0.10	376.0	271.1	67.5	54.0	142.7	107.8	117.8	137.3	166.7	183.0	110.4	158.7	113.7
	0.01	177.0	560.5	44.2	95.7	138.0	135.0	93.8	87.3	96.7	98.8	86.4	136.7	103.0
Naphthalene acetic acid	0.10	193.2	286.8	47.0	120.6	458.8	681.0	50.0	257.0	840.0	58.3	181.8	790.0	95.2
	0.01		1210.5		351.0		1673.2	257.8		1178.3	232.1		565.6	246.9
Indole acetic acid	0.10	135.8	560.5	28.7	63.5	324.7	669.3	249.6	102.8	451.7	177.8	75.3	128.6	105.5
	0.01	332.2	765.8	58.0	47.6	104.8	274.0	73.6	123.5	583.3	233.7	108.9	138.6	122.3

Nicotinic acid also has been reported to be required for the continued growth of excised roots (1) and to stimulate the growth of certain plants (2). Its effect on lettuce is similar to that of thiamin and of thiourea, but was toxic in concentrations of 10 and 1 mg. per liter.   A concentration of 0.01 mg. per liter produced definite stimulation of the growth of excised roots.

The inhibitory effect of auxins on root growth has been repeatedly reported in the literature although certain workers have found a stimulation.   This apparent discrepancy is perhaps due to the variation of experimental method, as well as to the variations in response of different species.
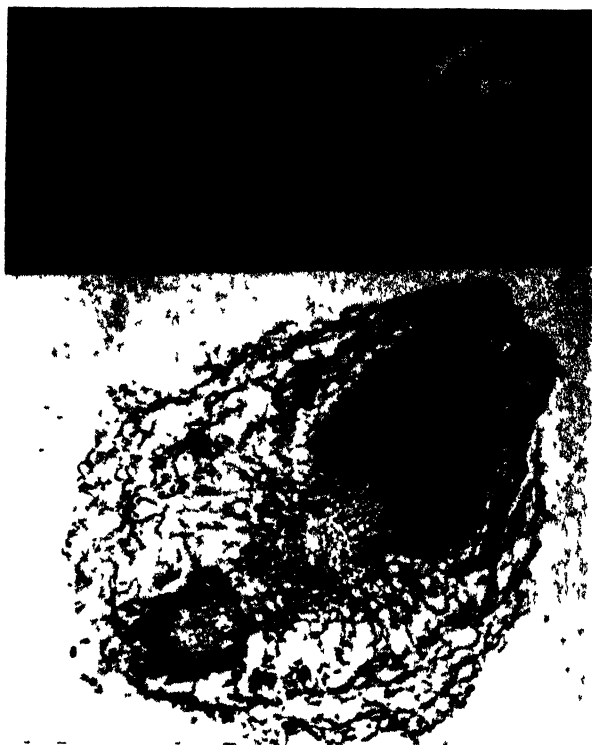
FIG. 8.   Whole plant cultured in nutrient plus ten milligrams per liter of naphthalene acetic acid, showing nodule like inhibited root primordia, and below, transverse section through one of them.

GEIGER-HUBER and BURLET (8) and FIEDLER (7) report increase of growth for the lower concentration under conditions similar to our experiments; BONNER and KOEPFLI, however, (4) report inhibition of root growth in Avena for the same concentration in which the greatest growth took place in these experiments.   Their experiments, however, lasted only over a 24-hour period.   It is possible that the stimulation which was observed here is due to a secondary effect since the culture solution at the end of the two weeks does not give curvature in the Avena test.   The auxin is therefore presumably either used up or in some way destroyed by the plant, but the

fact remains that the stimulation of growth is the result of addition of the auxin to the culture.

The auxins used in these experiments, indole acetic acid and naphthalene acetic acid, gave very similar results. The results, however, with naphthalene acetic acid were roughly comparable to those in ten times the concentration of indole acetic acid. No normal growth occurred in the ten-mg.-per-liter concentration of either auxin. Excised roots were completely inhibited in their growth and no nodular growths (inhibited primordia) were formed. The entire plant elongated but a few millimeters in this concentration. Thickening of the leaves occurred, and many nodular growths were formed
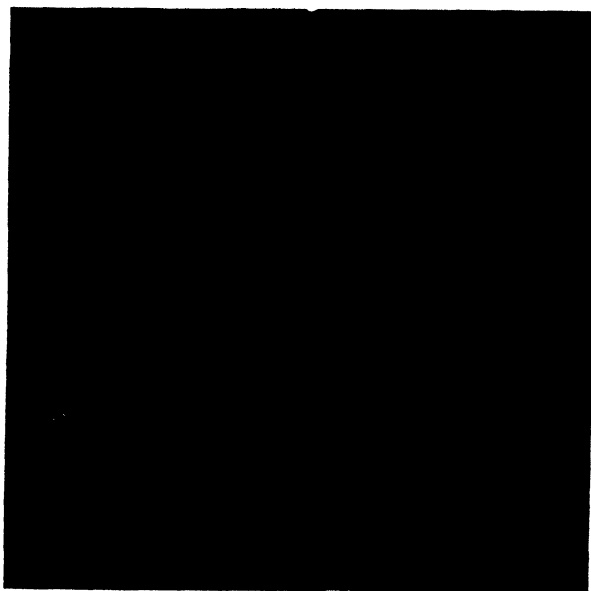
FIG. 9. Above: left, whole plant in nutrient only; center, plus 0.1 mg. per liter indole acetic acid; right, plus 0.01 mg. per liter naphthalene acetic acid. Below: left, upper, excised root in nutrient only; lower, plus 0.1 mg. per liter thiamin; center, plus 0.1 mg. per liter indole acetic acid; right, plus 0.1 mg. per liter naphthalene acetamide.

on both the root and the epicotyl (fig. 8). These nodules are evidently the result of the initiation of root primordia which are subsequently inhibited in their growth.

In the 1.0-mg.-per-liter concentration of auxins almost no growth takes place, but some primordia are initiated on the excised roots as well as on the shoots and on the intact plants. Those on the excised roots, however, are poorly organized and often resemble a cancerous growth more than root primordia. More primordia were initiated in naphthalene acetic acid than in indole acetic acid and subsequent inhibition was greater, resulting again in a nodule-like appearance as in the 10-mg.-per-liter concentration. The primordia on the shoot and on the intact plant developed somewhat giving rise to very short, thick, and profusely branched roots. No branch roots

developed on the excised roots. The comparative counts of numbers of primordia and lateral roots showed that there are more primordia formed on separate shoots than on shoots when the root is present; but the number on the roots is greater in the latter case than on the roots cultured separately, and also the number on shoots alone is greater than the sum of those initiated on both the roots and shoots cultured together.

Great stimulation of root growth occurred in all series in the 0.1- and 0.01-mg.-per-liter concentrations of both auxins (fig. 9). The length of root was not determined in the 0.1-mg.-per-liter concentration series of naphthalene acetic acid since there were a very great number of exceedingly short, thick roots whose linear measurement was almost impossible to determine, and, even then, would have been without significance. Swelling of the cortex also occurs and is occasionally so severe that the outer layers of the cortex are completely separated from the central cylinder. Roots were in all cases much thicker than those of control plants.

Summary

In an effort to approach simultaneously the problems of growth correlation and the effects which certain reported growth regulatory substances exert upon young seedlings, the author utilized aseptic tissue culture technique methods to compare four series of cultures: (1) intact seedlings, (2) excised roots, (3) excised shoots, and (4) a combination of excised roots and excised shoots. In these four series, thiamin, thiourea, nicotinic acid, naphthalene acetamide, naphthalene acetic acid, and indole acetic acid were added to the culture medium of inorganic salts and sucrose in concentrations from 0.01 to 10 mg. per liter. The control group (without added substances) showed that excised root growth was slightly benefited by the presence of the shoot in the medium, that the growth of adventitious roots on the excised shoot normally far exceeded that of the roots on the intact seedling, and that a further slight stimulation of this growth of adventitious roots on the excised shoot occurred when the excised root was present in the medium. When this group was compared with those obtained for the various growth substances in the lower concentrations, certain striking differences were seen. In thiamin the further stimulation by the excised root of the adventitious root growth on the excised shoot did not occur. Neither did it occur in nicotinic acid. Also the characteristic slight increase in growth of the excised root taking place in the presence of the shoot did not occur in nicotinic acid. It appeared that both of these substances, then, disturbed the usual reciprocal relationship between the root and the shoot as found in the control group. Thiourea did not appear to alter this relationship, which was affected to a greater extent, although differently, by naphthalene acetic acid. Naphthalene acetic acid brought about an increase in the growth of excised roots alone, both over the control, and over those also in the naphthalene acetic acid, but in the presence of the shoot. This is directly opposed to the relationship of the two excised root series in the

control group. Similarly, the increase in root growth on the intact seedling brought about by naphthalene acetic acid overshadowed the response of the adventitious roots on the excised shoot, which in turn was greater when the shoot was alone than when it was in the presence of the excised root. This again was exactly the reverse of the situation in the control group. The same type of response was noted with indole acetic acid. It was also found in the response of excised roots to naphthalene acetamide, but the growth of roots on all three series of shoots in this substance followed the trend of the control group. The striking difference between the response of the different parts of the plant to the acid and the amide of the same substance should be noted.

Various morphological irregularities were observed in the higher concentrations of some of the substances.

The author believes that problems concerned with the effects of growth regulating substances on the plant must be attacked simultaneously with those on growth correlation, or any conclusions derived therefrom will be seriously limited in their scope.

LITERATURE CITED

1. Addicott, F., and Bonner, J. Nicotinic acid and the growth of isolated pea roots. Science n.s. **88**: 577–578. 1938.

2. Bonner, D., and ——————. On the influence of various growth factors on the growth of green plants. Amer. Jour. Bot. **27**: 38–42. 1940.

3. ——————, and Greene, J. Vitamin B_1 and the growth of green plants. Bot. Gaz. **100**: 226–237. 1938.

4. ——————, and Koepfli, J. B. The inhibition of root growth by auxins. Amer. Jour. Bot. **36**: 557–566. 1939.

5. Bonner, J., and Axtman, G. The growth of plant embryos *in vitro*. Preliminary experiments on the role of accessory substances. Proc. Nat. Acad. Sci. **23**: 453–457. 1937.

6. ——————, and Addicott, F. Cultivation *in vitro* of excised pea roots. Bot. Gaz. **99**: 144–170. 1937.

7. Fiedler, H. Entwicklungs- und reiz-physiologische Untersuchungen an Kulturen isolierte Wurzelspitzen. Zeitschr. Bot. **30**: 385–436. 1936.

8. Geiger-Huber, M., and Burlet, E. Über den hormonalen Einfluss den B-Indolyessigsäure auf des Wachstum isolierter Wurzeln in Keimfreier Organkultur. Jahrb. wiss. Bot. **84**: 233–253. 1936.

9. La Rue, C. D. The growth of plant embryos in culture. Bull. Torrey Bot. Club **63**: 365–382. 1936.

10. Robbins, W. J. Cultivation of excised root tips and stem tips under sterile conditions. Bot. Gaz. **73**: 376–390. 1922.

11. White. P. R. Plant tissue cultures. A preliminary report of results obtained in the culturing of certain plant meristems. Arch. Exp. Zellf. **12**: 602–620. 1932.

12. ——————. Potentially unlimited growth of excised tomato root tips in a liquid medium. Plant Physiol. **9**: 585–600. 1934.

13. ——————. Plant tissue cultures. Bot. Rev. **2**: 419–437. 1936.

14. ——————. Potentially unlimited growth of excised plant callus in an artificial nutrient. Amer. Jour. Bot. **25**: 18s. 1938.

EFFECT OF THE ROOT SYSTEM ON TOMATO STEM GROWTH

F. W. WENT

(WITH TWO FIGURES)

A long succession of observers have found a correlation between elongation of internodes of the stem axis and the presence of the stem tip. Botanists are in general agreement at present that this correlation may be attributed to the formation of auxin in the stem tip and its transmission through the stem to the elongating regions. Shoot growth is also dependent upon factors supplied by the root system. Vigorously growing branches soon suspend growth in length after they have been cut and placed in a vase. This root influence on shoot elongation is not due to the better known functions of the root system, namely water and salt uptake, for no set of conditions insuring adequate water and salt supply of the cut shoot can replace the loss of the root systems. In several papers (**14, 15, 17**) this effect of the root system on growth has been investigated in some detail and definite indications of the substantial nature of this effect have been presented. An analysis of the distribution of growth rates in the Avena coleoptile also led to the assumption of a second growth factor in addition to auxin required for stem elongation. In earlier papers this factor was non-committally named "food factor" (**12, 13**), but later when it became evident that sugar was not identical with this "food factor" (**9, 10, 17**) a special name, caulocaline, was used for this second factor without any commitments as to its nature.

In experiments with pea seedlings the root system was found to exert its specific effect on shoot growth even when it did not have to take up nutrients, and the effect was most pronounced when the roots were in contact with the minimum amount of water. If the roots were submerged too far in the non-aerated tap water, shoot growth was much decreased (**15, 17**). BONNER and AXTMAN (**3**) and SKOOG (**11**) found that in excised embryos the presence of growing roots increased shoot growth. This is remarkable because one would rather expect the shoots to be in food competition with the roots.

All this evidence leads us to the hypothesis that under proper conditions the root system produces a hormone, caulocaline, which is required for stem growth in conjunction with auxin and sugar (**17**). In the present paper the conditions under which the root system exerts its influence on stem growth was studied. All work was done with tomatoes grown in the greenhouse.

Methods and results

To analyze the different functions of the root system, young tomato plants, San José Canner variety, were grown in sand. When they had reached a length of 10 to 15 cm. the root system was washed free of the adhering sand, and the stem below the cotyledons was split lengthwise so that on one

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plant two separate root systems were obtained, each attached to one half of the stem base. The plants were then placed over two adjoining containers with HOAGLAND nutrient solution (6) so that half of the root system dipped into each container. The nutrient solution in each container was aerated, and within two weeks the root systems were well developed. Then the two halves of the root system could be subjected to different conditions in an attempt to separate its various functions. As an example, one of the first experiments will be described.

The plants were divided into three groups of 5 to 10 plants each. Group A remained with both portions of the root systems in nutrient solution. Group B consisted of plants in which one-half of the root system was killed, so that they had only one-half of the functional root system in the culture solution. In group C the nutrient solution around the one-half root system was left, but around the other half it was exchanged for peat, which was kept moist with tap water.

Figure 1 indicates the rate of stem elongation of the three groups. Be-
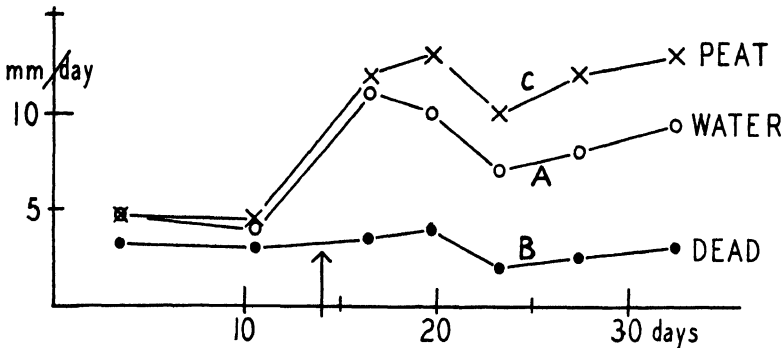
FIG. 1. Growth rate (ordinate, mm./day) of stems of tomato plants with split stem bases and root systems. Both halved root systems of each plant are submerged in nutrient solution, until the 14th day (arrow), when one half is left in the solution, and the other half is either transferred to peat (C), or is left in solution (A), or is dead (B).

fore transfer of roots of C, groups A and C had the same growth rate. In those plants the root system was already close to limiting the growth rate, for plants with only one-half living root system grew less (group B).

Four days after transfer of group C its growth rate was still approximately the same as that of A, but in subsequent periods the growth of the former became and remained significantly above that of A in spite of the fact that the root system effective in taking up salts and water was reduced to one-half. The increase in growth rate followed the appearance of many new roots with abundant root hairs on the root system in peat. Observation showed that relatively little water was taken up from the peat, the bulk coming from the nutrient solution.

In this and later experiments it was noted that even with abundant aeration of the nutrient solution, chlorosis developed in the tomato plants having all roots submerged in the solution. This chlorosis became especially

severe when the pH of the nutrient solution was approximately 7, and it was less pronounced at pH 5 to 6; even at this lower pH the plants were only light green. Even the severest chlorosis disappeared, however, as soon as roots developed in the peat, or above the nutrient solution. This might have been due to improved uptake of iron from the peat, since iron humates are known to be present in peat and to be an excellent source of iron for the plant. For this reason, an inorganic inert medium was compared with peat. For this inorganic medium ⅛- to ¼-inch-mesh haydite was chosen, a pumice-like, light-weight, inert, burned-shale, sharp-edged, material which can hold a considerable amount of water and gives aeration as good or better than peat. Four groups of 5 tomato plants each were set up with halved root systems. The growth rate of the groups was comparable and almost constant for a period of two weeks as shown in table I. After transfer of one-half the root system to the solid medium, the growth rate of these plants almost doubled one week after the transfer whereas the growth rate of the plants with both root systems in solution fell off to a very low rate.

This same type of experiment was repeated at least ten times, always with the same results. When the pH of the nutrient solution was kept at 5, the growth of the plants with both root systems in solution was better than at a higher pH, but it was always exceeded by the plants with one portion of their root systems not submerged in solution. This fact is stressed by the experiment shown in table II, where the growth rate of the plants with one portion of the root system in silica gravel failed to increase above that of the control plants. This was due to the fact that for the first 13 days after transfer to gravel the water level was kept up to the surface of the gravel. Upon draining of the gravel the growth rate immediately increased. In this case, a pure quartz gravel washed for 5 hours with strong H_2SO_4, then leached with rain water for 24 hours was used. Its color did not indicate the presence of any iron. Still the plants with half of their roots in this material, watered with rain water, became dark green. When half of the root system of these plants was cut off, only those plants having roots left in the gravel continued to develop green leaves even though they had no roots left in the nutrient solution. All of these experiments show that the effect of roots growing outside the nutrient solution upon the formation of the green color of the leaves is neither through iron uptake nor the iron uptake of the other roots, but by making iron (and other elements) available for chlorophyll formation. These roots can even offset the bad effect of high pH in the nutrient solution.

A summary of the data on the growth of the stem from 7 experiments, involving 140 plants, and all giving the same qualitative results, is presented in table III. The growth rate of the control plants remained constant or dropped over a 25-day growing period. The growth rate of the treated plants rose immediately after transfer of a portion of their root system to a solid moist medium.

The problem was also attacked with a slightly different technique. In-

TABLE I

GROWTH RATE IN MM./DAY OF TOMATO PLANTS (3 TO 5 PER GROUP) WITH SPLIT ROOT SYSTEM, THE HALVES SUBMERGED IN DIFFERENT CONTAINERS WITH NUTRIENT SOLUTION*

GROUP	ROOTS IN CONTAINER NO.	LENGTH IN MM. MARCH 19, 1940	MARCH 19-25	MARCH 25 TO APRIL 2	APRIL 2, 1940 HALF ROOT SYSTEM IN NUTRIENT OTHER HALF IN	APRIL, 1940							LENGTH IN MM. APRIL 25, 1940
						2-5	5-8	8-11	11-15	15-18	18-22	22-25	
A	1 2	100	7.8	7.0	Peat	5.7	9.0	7.7	10.0	10.0	7.5	15.7	417
B	2 3	98	7.0	8.0	Nutrient	6.7	8.0	4.7	1.5	3.3	2.0	2.0	292
C	5 4	104	7.7	6.0	Haydite	6.7	7.7	14.7	15.0	12.0	12.5	14.7	497
D	4 3	104	6.0	6.5	Nutrient	6.7	9.3	6.7	3.0	1.7	1.2	0.7	294

* The second column shows that, e.g., each of groups B and D had one-half of their root system in container 3.

TABLE II

ROOT SYSTEM OF TOMATO PLANTS SPLIT ON MARCH 18, 1941. BOTH HALVES WERE AT FIRST IN NUTRIENT SOLUTION WITH ADDED IRON; PH OF SOLUTIONS WAS KEPT BETWEEN 5.2 AND 6.0. ON MARCH 29 ONE HALF OF THE ROOT SYSTEM OF 10 PLANTS WAS TRANSFERRED TO SILICA GRAVEL. UNTIL APRIL 11 THE GRAVEL WAS COVERED WITH RAIN WATER, WHICH WAS DRAINED OFF ON THAT DATE, LEAVING THE GRAVEL MOIST, WITH EXCELLENT AERATION

ROOT SYSTEMS	GROUP	GROWTH RATE IN MM./DAY					
		MARCH, 1941		APRIL, 1941			
		21-28	28-3	3-11	11-14	14-18	18-21
Both in nutrient solution	A	mm. 4.8	mm. 5.9	mm. 11.2	mm. 7.7	mm. 10.6	mm. 7.0
One in nutrient solution, the other transferred to gravel on March 29	B	5.0 (One half root system in gravel)	10.7	10.6 (Water drained off gravel)	10.7	13.1	8.3
Growth of group B in percentage of group A		104.0	95.0	95.0	139.0	124.0	119.0

TABLE III

A SUMMARY OF STEM GROWTH (FOR 3.5-DAY PERIODS IN MM./DAY) OF TOMATOES GROWN
WITH BOTH PORTIONS OF THEIR HALVED ROOT SYSTEMS IN NUTRIENT SOLUTION
(TOP ROW).   MEAN OF SEVEN EXPERIMENTS, EACH COMPRISING 20 PLANTS

	OBSERVATION PERIOD IN DAYS						
	3.5	3.5	3.5	3.5	3.5	3.5	3.5
Both portions of the root system in nutrient*	8.8	8.5	8.0	8.8	9.0	6.3	6.4
One half root system in nutrient, other half transferred to solid medium after 3rd period	8.6	8.3	7.7	11.1	12.4	10.8	9.9
Growth of treated group in percentage of control group	98	98	97	129	138	170	154

* Figures in second row refer to plants 10.5 days before and 14 days after one portion
of their root system was transferred from nutrient solution to either moist peat, haydite,
silica gravel, or glass wool.

stead of mechanically dividing the root system into two parts, tomato plants
were induced to develop a root system outside the nutrient solution in addi-
tion to the roots in the nutrient.   This was done by growing tomatoes in wire
baskets containing a layer of about 3 cm. of peat, haydite, gravel, sand, or
soil, which was kept wet with tap water.   These baskets were suspended
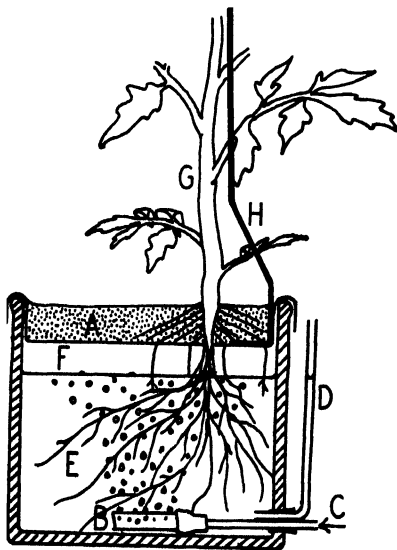
FIG. 2.   Cross section through a two gallon earthenware crock, two-thirds filled with
nutrient solution (E), through which air (C) is finely divided with aerator (B).   The
water level can be read at D.   On top of the crock is attached a wire basket, filled with
peat (A).   In this peat a tomato plant (G), tied to support (H), develops a root crown,
and in the solution the feeder roots branch out.   F is air space between peat and nutrient
solution.

from the edge of two-gallon crocks, two-thirds filled with nutrient solution, which was well aerated (fig. 2). The stems of tomatoes, germinated in sand and transplanted into the baskets, extended through the wire basket so that all roots dipped into the nutrient solution, and within two weeks from planting, a second root system developed in the medium in the basket. In general, those roots remained short and had many root hairs, but occasionally some of them grew down into the nutrient solution and then elongated very much.

Under these conditions the tomatoes grew slowly until the roots in the basket were well developed, then their growth increased to approximately the same rate as that of tomatoes grown in sand and watered with nutrient. The following growth rates in mm. per day were measured for plants approximately 300 mm. tall in a humid greenhouse (70 to 80 per cent. humidity, 26.5° C.) ; 14.6 with haydite in basket; 14.3 with peat in basket; and 16.1 in ordinary sand culture. In the dry greenhouse (30 to 40 per cent. humidity, 26.5° C.) 11.6 with haydite and 10.2 with peat. In other instances growth rates as high as 27.2 mm. per day were measured, which compared with 26.9 for similar tomatoes grown in gravel with sub-irrigation (both with day temperatures of 26.5° C. and night temperatures of 20° C.). The aeration of the solution is of no importance for the growth of the tomato plants as soon as roots have developed in the basket. In some experiments it was even found that aeration decreased the growth rate in direct proportion to the amount of air passed through the solution. In 16 non-aerated plants the growth rate was 11.1 mm. per day. With an air stream of 0.5 to 2.5 ml. per minute the growth rate was 9.0; from 16 to 17 ml. per minute it was 8.6; from 25 to 60 ml. per minute it was 8.4, and from 100 to 180 ml. per minute it was 7.1. In another experiment 18 tomato plants with their roots in an aerated nutrient solution grew at a rate of 22.9 ± 1.6 mm. per day over a two-week period, whereas 16 comparable plants, in unaerated solution, grew 26.3 ± 1.0 mm. per day. This same difference was maintained in following weeks. The standard deviation in the aerated plants was in every case higher than in non-aerated plants. This was due to a much greater number of plants with extreme growth rates, mainly with extremely low rates.

In the plants in the baskets chlorosis also developed when the pH of the culture solution was above 6 and when no roots had developed in the medium in the basket. As soon as the roots grew out in this medium chlorosis disappeared. Sometimes only half of the plant became green and in these cases it was found that roots had developed only at the side of the stem below the green sector. This localized effect was also observed in the tomatoes with the halved root system described before. Here also the sector of the tomato plant above the root system in the solid medium turned green and only much later the whole plant changed color. Another indication that the nutrient solution as such is not responsible for the chlorosis was found in the observation that two plants in the same basket with their roots

TABLE IV

GROWTH RATES IN MM./DAY OF PLANTS GROWING UNDER DIFFERENT CONDITIONS, AND THE GROWTH RATES OF THESE SAME PLANTS AFTER THE ROOTS DEVELOPED IN HAYDITE OR SOIL IN THE BASKET HAD BEEN CUT OFF

| | | GROWTH RATE IN MM./DAY | | | | | | | | |
| | BASKET FILLED WITH | ORIGINAL LENGTH APRIL 18 | APRIL | | | | MAY | | | |
			18–21	21–25	25–28	28–5	5–9	9–12	12–16	16–19
Solution aerated	haydite	24.1	5.2	7.9	10.5	16.0	20.7	19.7	18.7 / 11.2*	18.7 / 10.0
Solution not aerated	haydite	28.5	4.7	9.7	19.7	20.4	27.2	28.3	21.6	21.6
Solution not aerated	soil	25.0	5.7	9.6	24.1	24.1	29.2 / 27.0†	27.3 / 18.3	26.9 / 19.2	26.9 / 17.7

* Roots in basket cut off May 12.
† Roots in basket cut off May 7.

in the same solution might be very different. The one with roots in the peat, sand, gravel, or haydite was dark green and grew rapidly, whereas the one without a root system outside the nutrient solution was yellowish green and remained stunted.

That the growth rate of the plants in these baskets is in the first place determined by the roots developing in the medium above the culture solution was indicated by the fact that the growth rate of the plants remained low as long as the roots in the basket had not developed. The complementary experiment in which the roots growing in the basket were being cut off gave the expected result (table IV). In another experiment, within a week after cutting the roots in the basket, the growth rate of the tomatoes had dropped to one-third of that of the controls in spite of the fact that the dry weight of these roots, which were removed, was much less than 10 per cent. of the dry weight of all roots. By cutting off four-fifths of the root system which developed in the solution the growth rate temporarily dropped to about 50 per cent., but soon returned to normal. This indicates that less than ten per cent. of the root system in those tomatoes is responsible for more than 50 per cent. of their growth rate.

It would be expected from the previous experiments that there is a rather close correlation between the growth rate of the tomato stems and the weight of the root system developed in the basket. In an attempt to determine whether this was due to this root system as such or to other factors a number of determinations of the sugar content, auxin content, osmotic concentration of the cell sap, etc., were carried out in two sets of plants which had shown great difference in growth rate. One set of plants had been growing in a green house maintained day and night between 26° and 27.5° C. and 30 to 40 per cent. humidity; the other set was grown under exactly the same conditions except that the humidity was kept between 70 and 80 per cent. Under these conditions tomato plants grown in gravel with sub-irrigation showed exactly the same growth rate (for a weekly period in the dry house 21.5 mm. per day, in the wet house 21.5 mm. per day). The difference in growth rate of the two sets of plants grown in baskets above nutrient solutions, therefore, was not due to the different humidities as such. At the low humidity the peat in the basket dried out more rapidly and therefore had decreased the development of roots. In table V a number of the determined values have been condensed. These figures show a great difference in growth rate of the stems between the tomatoes grown in the dry and in the wet atmospheres. They also show that the difference is not correlated with sugar content of leaves, leaf development, root development in the nutrient solution, osmotic concentration and pH of the roots and stem tissues, auxin content of the green tips, or thiamin content of leaves, tips, and roots, for they all are of the same magnitude in the two groups of tomatoes with the different growth rates. Under wet conditions the stomata were more open, and the suction force was slightly less; but these same differences were found in the plants under sub-irrigation which did not show any differences in

growth rate.   The only outstanding difference between the two sets of plants was the weight of the roots in the basket and stem length, weight, and growth.

The root system in some solid, well-aerated medium is not essential for growth.   Even when the whole root system is completely submerged in nutrient solution growth takes place, although in the author's experiments at a decreased rate.   If the iron content of the nutrient solution is sufficiently high (10 to 100 times more than required when used in sub-irrigation) and when the pH is carefully kept adjusted, chlorosis does not necessarily develop in tomatoes which have their complete root system submerged

TABLE V

VARIOUS VALUES FOR TOMATOES GROWN IN BASKETS ABOVE NUTRIENT SOLUTIONS AT THE SAME TEMPERATURE AND LIGHT CONDITIONS BUT IN DIFFERENT RELATIVE HUMIDITIES.*   EACH VALUE IS THE MEAN OF 3 TO 8 DETERMINATIONS

	WET HOUSE	DRY HOUSE
Total length when harvested (mm.)	362	271
Growth rate in mm./day	24.7	13.8
Dry weight of leaves (mg.)	1518	1228
" " per leaf (mg.)	134	123
" " of roots in basket (mg.)	155	72
Wet weight of roots in solution (gm.)	6.75	6.47
" " " stems (gm.)	20.72	11.35
Osmotic concentration of press sap from roots (atm.)	4.82	4.46
" " " " " " stems (atm.)	8.19	8.07
pH of press sap from roots	5.52	5.50
" " " " " stems	5.20	5.20
Auxin content of tops in degrees curvature/gram	74	80
Vitamin B_1 in γ/gram dry weight of tops	14.3	12.0
" " " " " " " leaves	8.2	8.5
" " " " " " " roots in basket	6.8	6.5
Glucose; percentage dry weight of leaves	0.82	0.88
Sucrose " " " " " "	0.61	0.79
Suction force (atm.)	8.71	11.1
Opening width of stomata (10 = wide open)	3.8	1.9

* Wet house, 75 per cent.; dry house, 35 per cent.

in nutrient solutions.   A very small proportion of all roots if outside the nutrient solution and in a healthy condition both offsets unfavorable pH or low iron content of the nutrient solution and greatly increases the growth rate.   Even roots which have developed in the saturated atmosphere above a nutrient solution can perform this function.

Only very few experiments were carried out to investigate whether the results obtained with tomatoes applied to other plants as well.   With Cosmos very striking effects were observed.   When young plants 8 cm. in length were transplanted in the peat baskets with their roots in the well-aerated nutrient solution, some growth occurred; but within 1 to 2 weeks the newly formed leaves were practically white, growth came to a standstill, and the completely etiolated tops started to die.   In a few plants this condition im-

proved again, and in all such plants roots were found which had developed above the nutrient solution or in the peat.   Increasing the iron and minor elements in the culture solution did not give the slightest improvement, whereas the same solution produced good growth when used to water Cosmos plants growing in sharp washed river sand or pure quartz sand or haydite.

The same effects were noted when Cosmos plants were grown suspended in jars with nutrient solution.   The stems were kept in position with a cotton plug, in which no roots developed.   Aeration of the culture solution did not give any improvement of the poor growth, and could not offset the chlorosis which developed both in aerated and unaerated solutions.   Within one week after lowering the nutrient solution to 6 cm. below the top of the jars the growth became normal again, but only in those plants which had a sufficient number of young roots developed in the air above the nutrient solution.   Also in this case the stem growth rate was determined by the extent of root development outside the nutrient solution.

Discussion

It seems that the previous experiments are sufficient to draw the following conclusions: A tomato plant with all of its roots submerged in a complete nutrient solution will grow slowly and may develop a chlorosis which cannot be cured by increased doses of iron and minor elements, even when sprayed on the leaves.   Aeration of the solution improves the development of the roots, but aeration itself cannot cure the condition of stunted growth and chlorosis.   This poor growth is *not* a result of insufficient water or salt uptake; at no time was wilting of plants observed.   From table V it follows that the sugar and the osmotic concentration of plants growing slow and fast was the same, so that apparently their salt concentration was also the same.   This is brought out more clearly by the experiments with divided root systems.   The plants do not become normal and healthy before roots develop outside the solution.   But then half the root system in solution is sufficient to take up all the water and salt required for good growth, whereas, beforehand double this amount of roots seemed insufficient.   The effect is so marked and appears so soon after transfer of the roots that an indirect effect of the roots in air on those in solution seems highly improbable. Effects due to better aeration of the root system in water through oxygen supplied by the roots in air are excluded since (1), aeration of the solution decreases rather than increases top growth; (2), the two portions of the root system are separated by 10 cm. of split hypocotyl, and these halved hypocotyls do not show development of aerenchyma.   Therefore, we must conclude that the portion of the root system in solution was perfectly capable of taking up all necessary salts and water, but that the top was unable to utilize them without the help of roots in air.   The experiments described above have shown that although the roots in the solid medium are able to take up water, the bulk of the water uptake occurs by the roots in nutrient solution.   Since the roots outside the nutrient solution have practically no

salt uptake, and cause increased growth even though they cannot take up organic materials (when grown in haydite, silica gravel, or sand), their effect can only be due to internal secretion of a factor required for satisfactory top growth. This same conclusion has been reached in the case of seedlings (14, 15), and this factor was named caulocaline. It is possible that caulocaline is a complex of factors; for further discussion the reader is referred to WENT and BONNER (17), where evidence of the chemical nature of caulocaline is produced. If we piece all present knowledge together, we can conclude: Roots supply a factor (or factors) to the growing region of the shoot, indispensable for stem growth, and for convenience sake named caulocaline. In many plants this caulocaline is formed only in roots surrounded by moist air. It travels upward in the stem, apparently under the influence of auxin (16), through the living elements of the vascular bundles (5) and has not been extracted in large quantities as yet.

Let us ask whether this knowledge about the formation of caulocaline is useful in explaining other well-known phenomena. In the first place, we have to bear in mind that the individual differences of various plants are enormous as far as the air requirement around their roots is concerned. Many plants such as rice, *Ranunculus sceleratus*, and *Cyperus alternifolius* (2) can grow with all of their roots submerged; but others, like tomato, must have part of their root system in contact with air to produce maximal growth. GERICKE (4) specifically mentions that in roses "the root crown should never be immersed in the liquid solution." This excessive aeration of the root crown is *not* required because otherwise no salts and water can be taken up; the oxygen requirement of the roots for salt absorption is much less than that for increasing the growth rate of the stems and for preventing the type of chlorosis described above.

Many plants require a very light and loose top soil. If the upper soil layers are allowed to pack closely together, growth in these plants is stunted. Although in most plants the roots, especially those taking up water and salts, are located deep down in the closely packed soil, still a superficial cultivation of the soil around such a plant will decrease growth if the superficial roots have been injured. This must be due to the necessity of the root crown for growth, because this cultivation does not appreciably change the conditions around the absorbing roots which are in the main below the cultivated portion of the soil.

The knee-roots, or pneumatophores, of the mangrove vegetation have long been considered to serve for air intake and gas exchange in general between the roots down in the mud and the air (7). Although it was physically impossible to get any considerable amount of gases exchanged over such a long distance (only through diffusion in the wide intercellular spaces of the pneumatophores) their respiratory function was generally accepted until TROLL and DRAGENDORFF (8) proved by direct measurements that no gas exchange of importance occurred through the pneumatophores of Sonneratia. It seems logical, therefore, to assume that these pneumatophores are necessary

for the caulocaline production required for stem growth. This view is strengthened by the observation of KARSTEN (7) that in the mangrove vegetation the trees with the largest pneumatophores have the largest growth rates.

In considering the bearing of caulocaline production on the growing of plants in general, water cultures have to be discussed. For 80 years plants have been grown with their roots immersed in nutrient solutions, and in the presence of all necessary inorganic elements satisfactory, although often slow growth was obtained. Proper aeration of the nutrient solution greatly increased growth in many plants. HOAGLAND and ARNON (6) have shown that with vigorous aeration tomato plants can grow as rapidly in water cultures as in good soil. GERICKE (4) suggested a modification of the water culture method consisting of supporting the plants above the nutrient solution in a seed bed containing some porous material, organic or inorganic. GERICKE's other improvements over the regular water-culture technique, such as the use of commercial salts and tap water, are adaptations of minor significance. A scientific explanation, however, is lacking for the advantages of hydroponics over the traditional water culture. Probably this is the reason why the importance of the seed bed is not generally recognized. In a pamphlet, BALL (1) states that hydroponics was no success in the East and Middle West, giving as the probable reason: "the Gericke plan furnished everything the soil did (see above) except air at the roots."

The experience gained with the foregoing experiments does not support the generally held views as expressed by HOAGLAND and ARNON (6): "While the use of a porous bed instead of a perforated cover facilitates aeration of roots, the bed can be dispensed with if provision is made to bubble air through the nutrient solution." This may be true for certain plants, but not for all. In a commercial greenhouse near Pasadena using the water culture method no other provision is made for aeration of the nutrient solution beyond pumping it slowly through the tanks. Since growth of tomatoes in this greenhouse is excellent, it indicates that aeration of the culture solution is not so essential if a proper root system has developed in the seed bed or between seed bed and culture solution.

We thus reach the conclusion that the essential improvement of hydroponics over the old water culture method is to divide the functions of the root system: one root system takes up water and salts; the other, in the seed bed or between solution and seed bed, supplies caulocaline. And this is essentially the same division of labor as we encounter in most trees and perennials. They also develop a long fibrous feeding root system, which penetrates deep into the soil, and in addition they have a root crown which is well aerated, but due to its position, cannot well serve for water and salt uptake.

Summary

It has been shown that if all the roots of a tomato (or Cosmos) plant be

submerged in a nutrient solution of pH 6 or higher, aeration cannot prevent chlorosis and especially a drop in the growth rate of the stems, although root growth is satisfactory. As soon as a portion of the root system develops in moist air, however, growth of the stem becomes maximal. All experiments point toward the conclusion that the part of the root system which develops in moist air supplies one or more factors (tentatively named caulocaline) required for stem growth and prevention of chlorosis. Thus, in intact plants, the aeration of roots seems to be of relatively greater importance for their caulocaline production than for salt uptake.

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LITERATURE CITED

1. BALL, V. Gravel crops. West Chicago. 1939.

2. BERGMAN, H. T. The relation of aeration to the growth and activity of roots and its influence on the ecesis of plants in swamps. Ann. Bot. **34**: 13–33. 1920.

√3. BONNER, J., and AXTMAN, G. The growth of plant embryos in vitro. Preliminary experiments on the rôle of accessory substances. Proc. Nat. Acad. Sci. **23**: 453–457. 1937.

4. GERICKE, W. F. The complete guide to soilless gardening. New York. 1940.

5. HAYWARD, H. E., and WENT, F. W. Transplantation experiments with peas. II. Bot. Gaz. **100**: 788–801. 1939.

6. HOAGLAND, D. R., and ARNON, D. I. The water culture method of growing plants without soil. California Agr. Exp. Sta. Cir. 347. 1938.

7. KARSTEN, G. Über die Mangrove-Vegetation in Malayischen Archipel. Bibl. Bot. Heft. 22. 1891.

8. TROLL, W., and DRAGENDORFF, O. Über die Luftwurzeln von Sonneratia Linn. f. und ihre biologische Bedeutung. Planta **13**: 311–473. 1931.

9. SCHNEIDER, C. L. The interdependence of auxin and sugar for growth. Amer. Jour. Bot. **25**: 258–270. 1938.

10. ————. The dependence of the Avena coleoptile growth-rate on the previous auxin supply. Amer. Jour. Bot. **27**: 711–718. 1940.

11. SKOOG, F. Experiments on bud inhibition with indole-3-acetic acid. Amer. Jour. Bot. **26**: 702–707. 1939.

12. WENT, F. W. Wuchsstoff und Wachstum. Rec. Trav. bot. néerl. **25**: 1–116. 1928.

13. ————. Coleoptile growth as affected by auxin, aging and food. Proc. Kon. Akad. Wet. Amst. **38**: 752–767. 1935.

14. ————. Specific factors other than auxin affecting growth and root formation. Plant Physiol. **13**: 55–80. 1938.

15. ——————. Transplantation experiments with peas. Amer. Jour. Bot. **25**: 44–55. 1938.
16. ——————. Some experiments on bud growth. Amer. Jour. Bot. **26**: 109–117. 1939.
17. ——————, and BONNER, D. Growth factors controlling tomato stem growth in darkness. (In press.)

USE OF TENSIOMETERS IN MEASURING AVAILABILITY
OF WATER TO PLANTS

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(WITH ELEVEN FIGURES)

Efforts by several investigators to measure availability of water to plants by vapor pressure (2, 12) and other methods (10, 14) have indicated that the potential of water at the permanent wilting percentage is approximately 16×10^6 ergs per gram, whereas others (1, 11) have obtained results which indicate somewhat lower values. A great many experiments (3, 4, 5, 6, 13) have demonstrated that soil moisture seems to be equally available to plants at all times, as measured by the response of plants, as the moisture content is decreased from a high value to about the permanent wilting percentage. This conclusion seems to be general for all plants which have been studied. Evidently plants do not respond markedly enough to show any change in availability of water until the soil moisture is reduced to about the permanent wilting percentage. On the other hand, physical measurements show that the energy required to remove water from the soil, changes materially as the soil-moisture content decreases. It was surprising therefore, when ROGERS (9) found, while working with strawberry plants in pots, that the plants wilted severely at a tension of 60 cm. of mercury (potential of 0.8×10^6 ergs per gram). Furthermore, his illustration shows plants wilted slightly at 47 centimeters (0.6×10^6 ergs per gram). The maximum potential measurable by tensiometers is less than 1×10^6 ergs per gram. The work reported herein was undertaken largely in an effort to explain this apparent discrepancy.

Experiments with sunflowers and strawberries

Since much of the work at Davis had been done with sunflowers, the first trial was made with these plants. The plants were grown in a metal container holding approximately 15 kilograms of soil. A tensiometer connected to a mercury manometer was inserted near the center of the container. The plants were allowed to dry the soil to the permanent wilting percentage several times before the experiment was started.

Figure 1 shows the tension as a function of average moisture content of the soil. The permanent wilting percentage and the moisture equivalent are given. The potential at the maximum reading of the gauge (56 centimeters of mercury) is approximately 0.8×10^6 ergs per gram. Figure 2 shows the appearance of the sunflowers when the tension was near the maximum value. Clearly the sunflowers were fully turgid; furthermore, they remained so until the permanent wilting percentage was reached. The gauge did not operate at the lower moisture contents. This disagrees with the results published by ROGERS (9). He worked with strawberry plants, however, so

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it was decided to see if by some chance these plants behaved differently than sunflowers. Strawberries were accordingly planted in double-walled burned clay jars (7), the inside wall of which was porous. The arrangement is shown in figure 4. Samples of soil to determine the moisture content in the jar were taken at various times with a cork borer, the holes being refilled with soil at approximately the same moisture content. The top half of the sample was kept separate from the bottom half and the average moisture content of the top half as well as the bottom half of the jar are plotted as a function of time in figure 3. The moisture equivalent and the permanent wilting percentage of the soil are shown. The plant continued to maintain its turgidity until the permanent wilting percentage was reached. Figure 4, A, was taken immediately following an irrigation so that all the
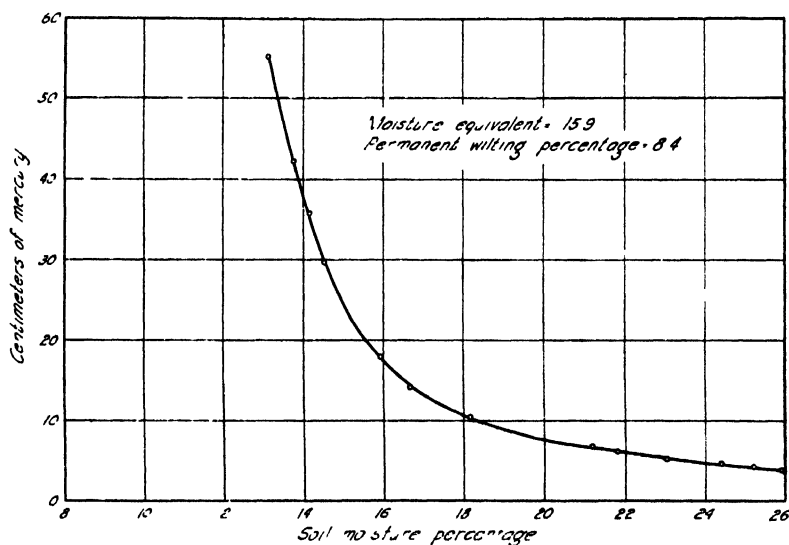
FIG. 1. Tension of the water expressed in centimeters of mercury as a function of moisture content for Yolo silt loam on which the sunflowers (292) were growing.

soil was wet and the gauge reading showed zero at the start of the experiment. Figure 4, B, shows the gauge reading 25 inches of mercury (potential of 0.8×10^6 ergs per gram) and the condition of the plant at this tension. There is no indication of wilting at this maximum tension read on the gauge.

As shown in figure 3, there was some difference in moisture content between the top half of the jar and the bottom half even though lead foil was used to cover the surface of the soil to retard evaporation. While there were variations in moisture contents in different portions of the soil, that at the center of the pot was not consistently higher or lower than at the edge. To test the distribution of tension within the jar a small porous cup was placed approximately in the center of the soil mass and connected to a gauge as shown in figure 5. The pot was then irrigated and the plants were allowed to reduce the moisture until the tension of 25 inches of mercury was

reached.   No corrections have been made for the difference in elevation of the gauges above the center of the porous vessel containing the water, but it is clear that the readings on the two gauges are approximately the same; that is, the tension at the outside wall is practically the same as in the center. This was to be expected from our measurements on the horizontal distribution of moisture within the pot as reported above.   Our results indicate that strawberries are no different from other plants studied in their ability to use soil moisture from soil permeated by their roots.   Our results, never-

FIG. 2.   Sunflowers (292) near the maximum obtainable tension of the water in the tensiometer.   They appear to be the same as the sunflowers (209) growing on soil above field capacity.

theless, differ markedly from those of ROGERS (9).   A possible explanation is that since ROGERS grew his plants in single-walled porous flower pots, evaporation from the wall had dried the outer part of the soil mass to a greater extent than the center of the pot where the porous cup used to measure the tension was located.

A further study was made of the use of the tensiometer with strawberries under field conditions.   The porous cup was connected to a tension gauge and placed with its center about 18 inches below the surface of the soil in a
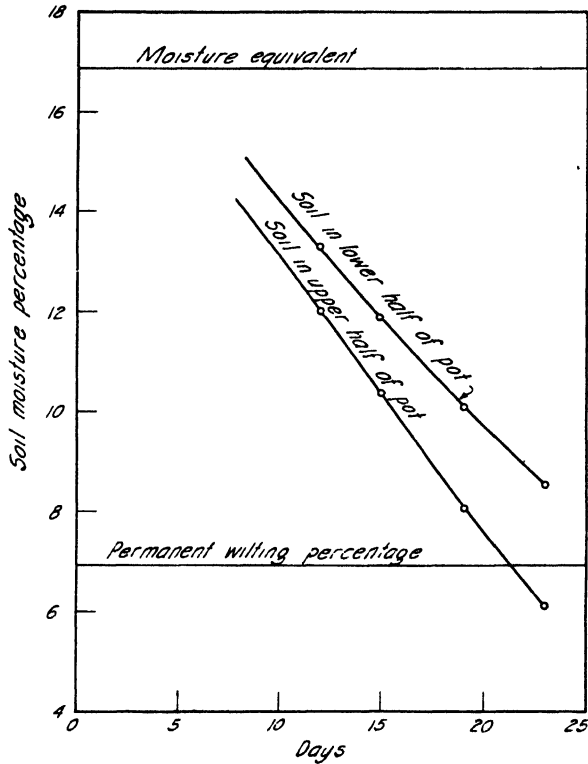
FIG. 3. Soil-moisture in the top half and bottom half of the double-walled pot shown in figure 4 containing Madera silt loam.

FIG. 4. Strawberry plant in a double-walled porous pot: (A) at the beginning of the experiment when the soil was wet; (B) the same plant when the tension of the water in the tensiometer had been increased to 25 inches of mercury. At this tension, there was no evidence of wilting.

strawberry bed.   Moisture samples were taken at 6-inch intervals to a depth
of 2 feet.   It is commonly thought that strawberries are extremely shallow-
rooted plants and, therefore, it was assumed that 2 feet would be ample
depth at which to sample.   Figure 6 shows four curves, one for each 6-inch
depth, giving the moisture content as a function of time.   The permanent
wilting percentage and moisture equivalent are also given.
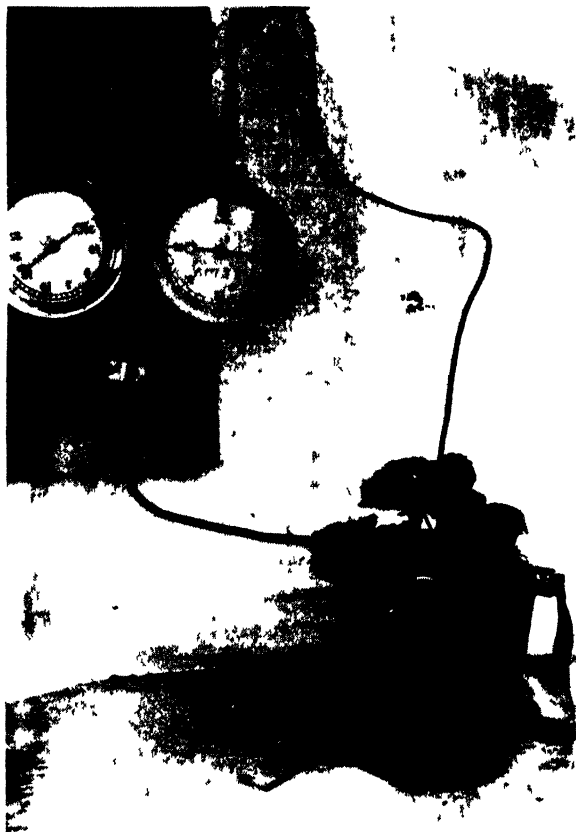
FIG. 5.   The readings of two gauges, one of which measures the tension of the water
between the two walls of the porous pot; the other is attached to a porous cup placed in the
center of the soil mass.   This arrangement was employed to determine whether there was
an appreciable difference in the tension between the center and the outside of the soil mass.
The readings were so close that no differences could be observed.

In figure 7 are shown photographs of the strawberries at the three dif-
ferent times indicated in figure 6 by the three vertical lines.   The picture
marked A was taken immediately following an irrigation.   The photograph
marked B shows the strawberries when they had dried the soil sufficiently
so that the gauge, which had been installed following irrigation, had reached
approximately 25 inches of mercury (potential of 0.8×10^6 ergs per gram),
no correction being made for the water column from the center of the cup
to the center of the gauge.   As the plants continued to use water, air grad-

ually leaked in so that the reading dropped to zero by the time the picture marked C was taken; at this time some of the plants were just beginning to show some wilting, although the maximum reading on the tensiometer had been reached 27 days previously. In fact, the plants continued to remain turgid after all the readily available water had been used out of the top 2 feet of soil. This was surprising in view of the fact that it is usually assumed that strawberries are shallow-rooted plants.

In an attempt to find out what the approximate root zone of the strawberry plant was, the bed was irrigated again and samples were taken at
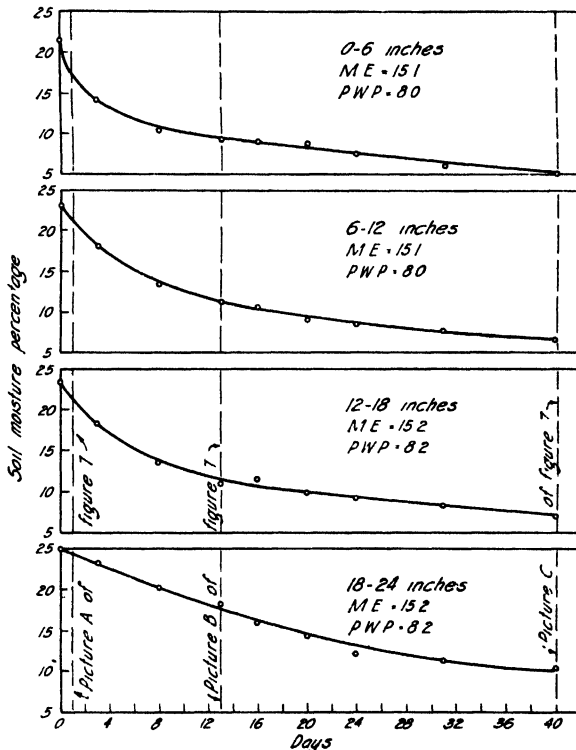
Fig. 6. Moisture content as a function of time for each 6-inch layer to a depth of 2 feet in the strawberry field shown in figure 7 on Yolo fine sandy loam. The three vertical lines show the times at which the photographs, figure 7, were taken.

1-foot intervals to a depth of 4 feet. The curves in figure 8 show the moisture content as a function of time for each foot section down to 4 feet. Apparently, some water was extracted below 3 feet. The conditions of the plants at various times are shown in figure 9. The picture marked A was taken immediately following an irrigation. The picture marked B was taken when practically all the available water was used from the top 2-foot section, whereas the picture marked C was taken at the end of the experiment. As can be seen, the plants show little need of water even at the end of the experiment. An appreciable amount of water was used from the 4th foot.

Replicability of tensiometer readings

Further studies on the use of tensiometers under field conditions were made on a Sudan grass plot. The tensiometers used here were constructed under the direction of Dr. L. A. RICHARDS (8), and, through his kindness, loaned to us for the experiment. Tensiometers were placed in the center of
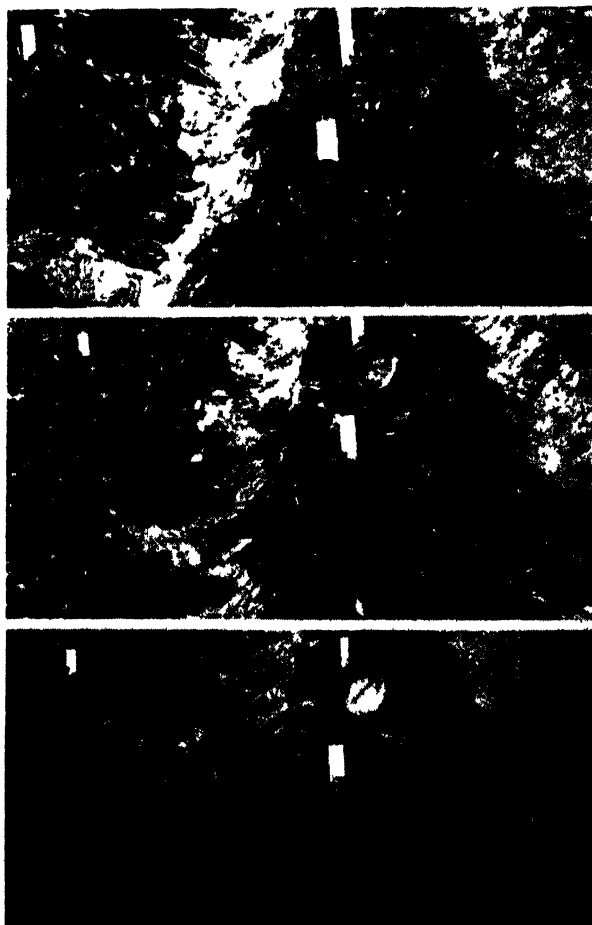
FIG. 7. (A) Strawberry plants immediately following an irrigation; (B) when the tension of the water in the porous cup attached to the gauge had reached approximately 25 inches of mercury; (C) when practically all of the readily available water had been used from the top 2 feet of soil. The tensiometer reading was approximately at its maximum value at the time B was taken. Air had leaked in and the gauge had dropped to zero by the time C was taken.

the first-, second-, third-, and fifth-foot depths. Variations of moisture content and tension with time for each depth are shown in figure 10, the tension being plotted to a logarithmic scale. The moisture equivalent and permanent wilting percentage are given for each depth.

At no time during the season did the Sudan grass show any indication

of need for water.   When irrigation was applied, it was done not because the grass showed need for water, but in an effort to start the field from a wet condition again so that a check on the replicability of the tensiometer could be made.   It should be mentioned that enough water was applied to this field to wet the soil to the water table 10 feet below the ground surface, and hence the soil below the top foot remained well above the moisture equivalent for a long period after the second irrigation.   To test the replicability of the tensiometer readings for different "cycles,"[1] the curves of figure 11 were derived from data taken from the curves of figure 10.   They show the tension as a function of moisture for each of the foot sections studied.
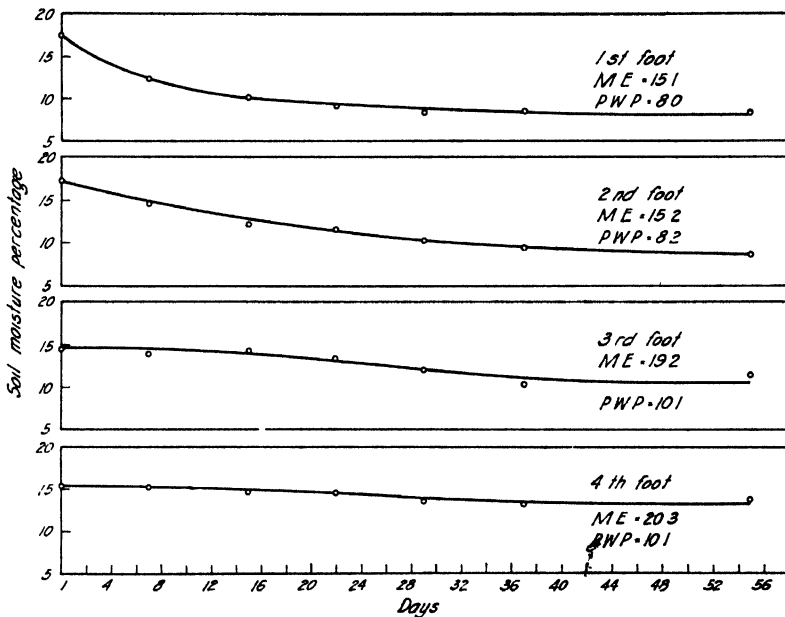
Fig. 8.   The soil-moisture content of the top 4 feet of soil in the strawberry field shown in figure 9.   This is the same field shown in figure 7.

The moisture equivalents for each foot are shown on the graph.   It is, of course, impossible to use the data for such a curve at tensions higher than where the tensiometer operates, and hence the moisture content does not decrease to as low a value as it does in the curves of figure 10.   The tension increases very rapidly as the moisture content decreases in the neighborhood of the moisture equivalent.   This makes it extremely difficult to fix with any degree of exactness the value of the tension at the moisture equivalent.   However, as can be seen from the curves of figure 11, a tension of 300 centimeters of water $(0.29 \times 10^6$ ergs per gram) is approximately the value when these soils are at the moisture equivalent.   This is in substantial agreement with RICHARDS (7).   It is considerably lower than most

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[1] By a cycle we mean the sequence of moisture changes which the soil passes through from one irrigation to another.
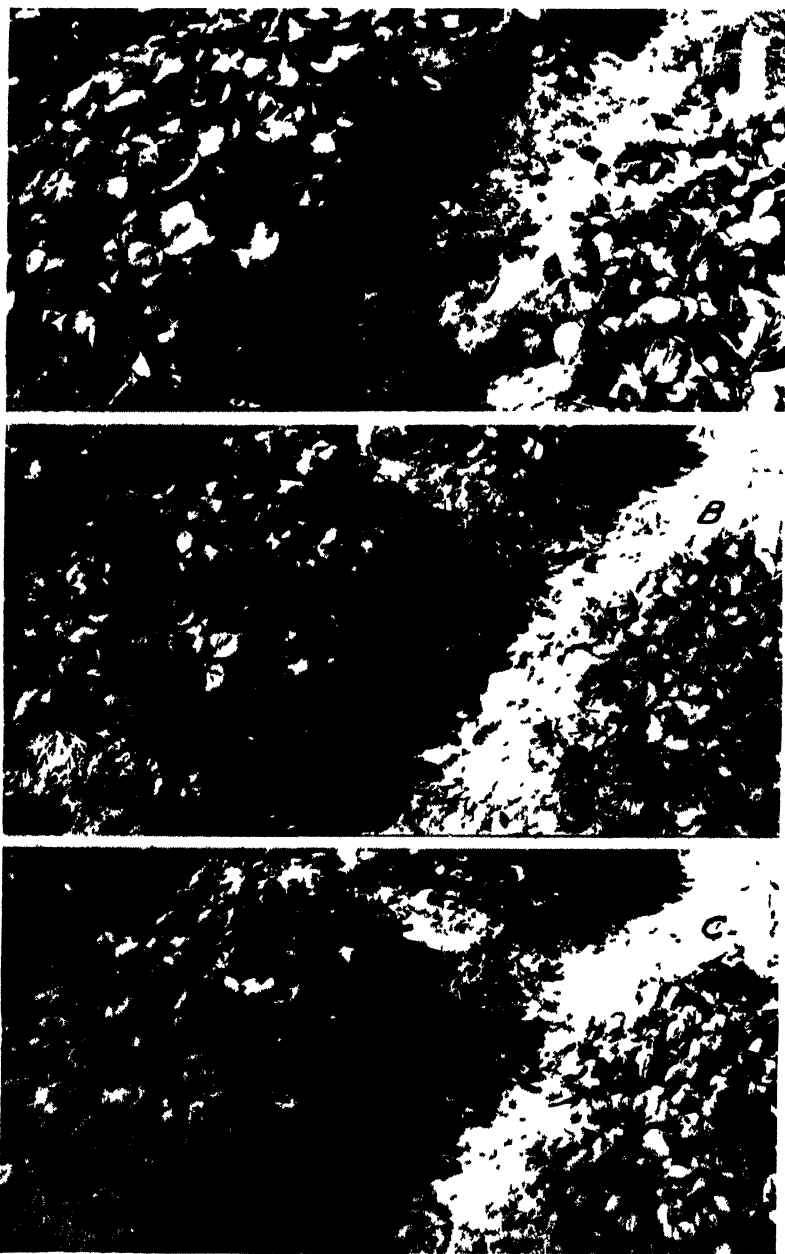
Fig. 9. (A) Strawberry plants immediately following irrigation; (B) 30 days after irrigation, and (C) at the end of the experiment 55 days after irrigation. The plants in B appear to be as turgid as in A.

of the values estimated by SCHOFIELD (10) who concluded from available data, that the pF was between 2.5 and 3.0 (0.31×10^6 to 0.98×10^6 ergs per gram) at the moisture equivalent.
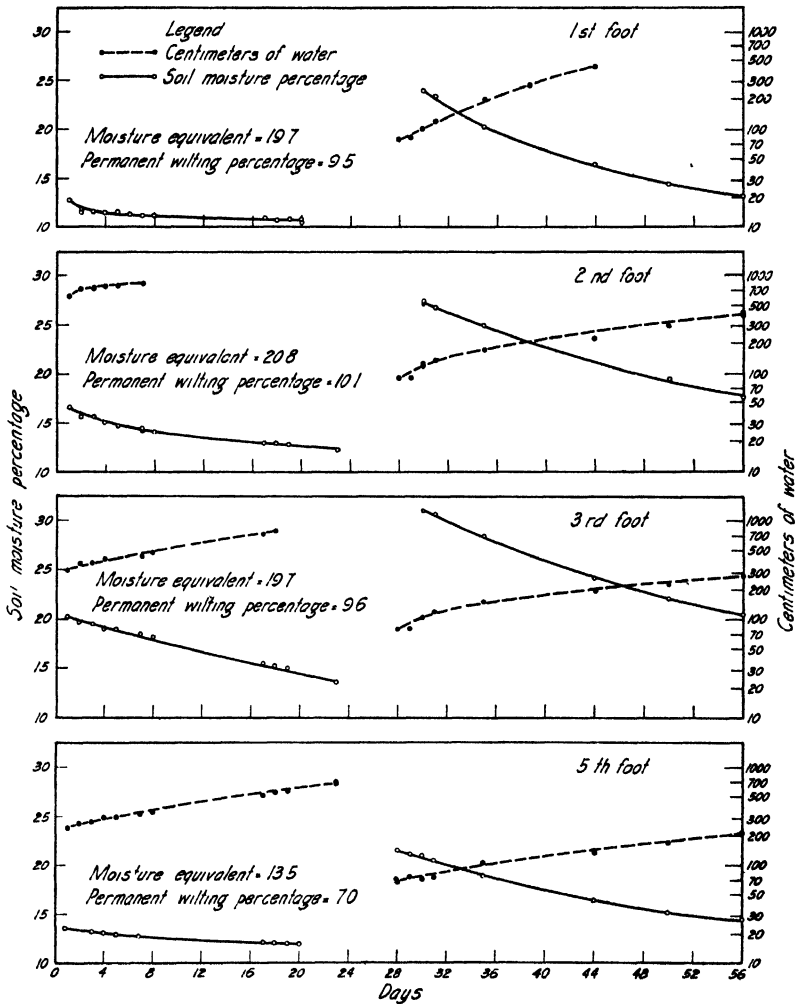
Fig. 10. The tension of the water in the tensiometers expressed in centimeters of· water and the moisture content in a Sudan grass plot as a function of time for the first, second, third and fifth-foot depths.

A confusion between numerical and dimensional equality in certain soil-moisture measurements

In this connection, it is worth while calling attention to an error which is frequently made. Since the moisture equivalent is approximately equal to the field capacity (which is usually considered to be the moisture held in the soil against the pull of gravity) of most fine-textured soils but not always sands, and since in the c.g.s. system the pull of gravity on unit mass is *numerically* equal to the pressure of 1 atmosphere, it has been reasoned by some that the potential at the moisture equivalent is equal to a tension of 1 atmosphere, or a pF of 3, or 1.0×10^6 ergs per gram. This is faulty reasoning. The fact that in the c.g.s. system the pull of gravity on unit mass is

approximately numerically equal to a pressure of 1 atmosphere is purely coincidental. The pull of gravity on unit mass has the dimensions of force per unit mass, whereas pressure has the dimensions of force per unit area; and potential has the dimensions of energy per unit mass. That pF of 3, or 1.0×10^6 ergs per gram is the value at the moisture equivalent has been
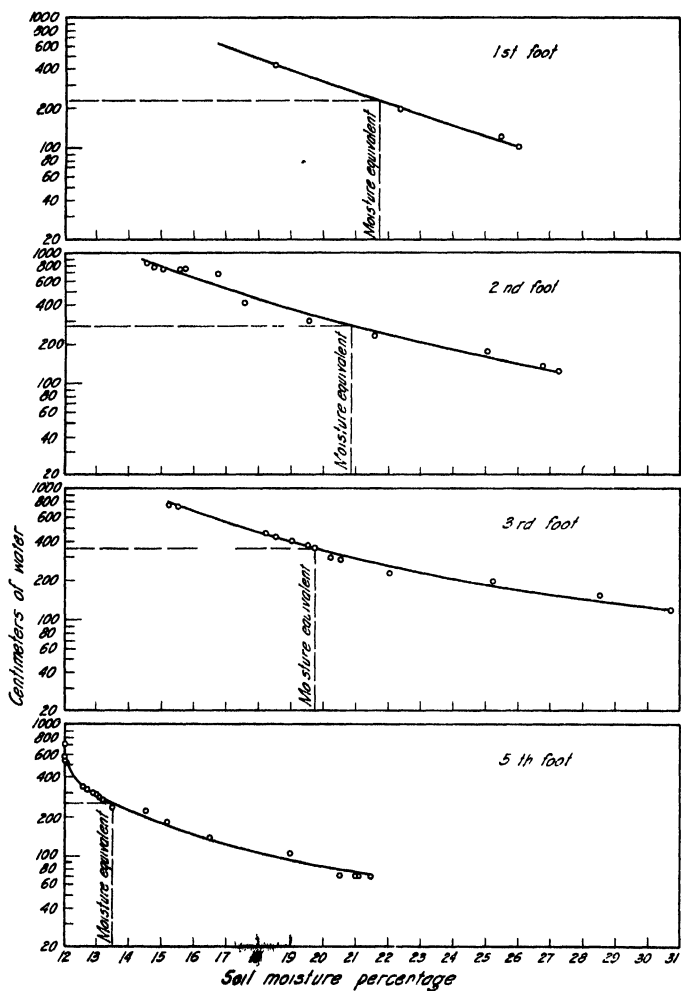
FIG. 11. The tension of the water in the tensiometers expressed as centimeters of water as a function of moisture content for the first, second, third, and fifth-foot depths in the Sudan grass plot, the moisture content of which is shown in figure 10. The tension at the moisture equivalent is indicated.

given added credence because it coincided with the upper range of values for pF at the moisture equivalent as estimated by SCHOFIELD. There is also another reason why a pF of 3 has been associated with the moisture equivalent. By assuming that a potential of zero exists at the outer surface of a soil sample one centimeter thick in a centrifuge being subjected to a centrifugal field of 1000 times gravity, it follows that the potential at the

inside surface is 1.0×10^6 ergs per gram or a pF of 3. The average pF for the sample is much less than this.

Summary

Pot and field experiments using sunflowers, strawberries, and Sudan grass indicate that tensiometers are capable of indicating the tensions for moisture contents in the upper one-third to one-half of the range between the moisture equivalent and the permanent wilting percentage.

Strawberry plants in pots remained turgid when the roots were in soil at moisture contents much lower than those which would produce a maximum tension of about 700 centimeters of water. Similarly, the moisture content in the top 3 feet of soil in the strawberry field reached a percentage lower than that which would show a tension of about 700 centimeters of water and yet the plants remained turgid.

All of the soil in the pots and in the top 3 feet of the strawberry field plots was reduced to the permanent wilting percentage before there was any evidence of wilting. At this moisture content there would be an equivalent tension of about 16,000 centimeters of water as estimated by available data on the potential of the water at the permanent wilting percentage.

Tensiometers in the Sudan grass plots showed close replicability for different "cycles." Tensions obtained were about the same even though the soils tested varied in texture as measured by the moisture equivalent.

For the soils tested, the potential at the moisture equivalent, while difficult to evaluate exactly, is in the neighborhood of 0.3×10^6 ergs per gram.

Attention is called to an error in reasoning occurring in the literature in regard to evaluating the pF and the potential of soil moisture at the moisture equivalent.

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LITERATURE CITED

1. BOUYOUCOS, GEORGE JOHN. The dilatometer method as an indirect means of determining the permanent wilting point of soils. Soil Sci. **42**: 217–222. 1936.

2. EDLEFSEN, N. E. A new method of measuring the aqueous vapor pressure of soils. Soil Sci. **38**: 29–35. 1934.

3. HENDRICKSON, A. H., and VEIHMEYER, F. J. Irrigation experiments with peaches in California. California Agr. Exp. Sta. Bull. 479. 1929.

4. ————, and ————. Irrigation experiments with prunes. California Agr. Exp. Sta. Bull. 573. 1934.

5. ————, and ————. Irrigation experiments with pears and apples. California Agr. Exp. Sta. Bull. 667. 1942.

6. ————, and ————. Readily available soil moisture and sizes of fruits. Proc. Amer. Soc. Hort. Sci. **40**: 13–18. 1942.

7. RICHARDS, L. A.   Uptake and retention of water by soil as determined by distance to a water table.   Jour. Amer. Soc. Agron. **33**: 778–786.   1941.

8. ——————.   Soil moisture tensiometer materials and construction. Soil Sci. **53**: 241–248.   1942.

9. ROGERS, W. S.   The relation of soil moisture to plant growth, illustrated by moisture meter experiments with strawberries.   Ann. Rept. East Malling Res. Sta. England. pp.111–120.   1935.

10. SCHOFIELD, R. K.   The pF of the water in soil.   Third Int. Congr. Soil Sci. Trans. **2**: 37–48.   1935.

11. SHULL, C. A.   Measurement of the surface forces in soils.   Bot. Gaz. **62**: 1–29.   1916.

12. THOMAS, M. D.   Aqueous vapor pressure of soils.   Soil Sci. **11**: 409–434.   1921.

13. VEIHMEYER, F. J. and HENDRICKSON, A. H.   Essentials of irrigation and cultivation of deciduous orchards.   California Agr. Ext. Cir. 50.   1936.

14. ——————, and ——————.   Some plant and soil-moisture relations.   Amer. Soil Survey Assoc. Bull. **15**: 76–80.   1934.

MEASUREMENT OF SMALL CONCENTRATIONS OF ETHYLENE AND AUTOMOBILE EXHAUST GASES AND THEIR RELATION TO LEMON STORAGE

P. W. ROHRBAUGH

(WITH FIVE FIGURES)

Introduction

Ethylene, even in very small concentrations, is known to hasten the coloring and ripening of fruits and vegetables in storage and to greatly increase their rate of respiration.

Further study of this problem has been made for the purpose of obtaining a satisfactory test for the detection of small concentrations of ethylene in connection with commercial storage of lemons. It was desirable to know what concentrations of ethylene accumulate in lemon storage rooms from decaying fruit; also what might be the importance of motor exhaust gases from loading and unloading trucks or from the cars and trucks of nearby highways.

Probably the earliest written record of the effects of motor gases on fruit is given in a paper by SIEVERS and TRUE (17). They reported that lemons were being colored quickly in the packing house by piping the exhaust gases from a gasoline engine into the coloring rooms. They also stated that the exhaust gases produced a very satisfactory fruit color in the same time as was required with kerosene stove fumes. Apparently the fruit was colored as quickly as it now is with ethylene.

WRIGHT (18) describes methods of using motor exhaust gases for coloring oranges. This author states that the gas in a well-regulated coloring room should be just strong enough that a person experiences only a slightly disagreeable odor or burning sensation of the eyes. His observation was that the first sensation experienced on entering the room is the most reliable. He recommends that the gas be run into the room during only about 10 hours a day.

BARGER and HAWKINS (1) have described the satisfactory use of gasoline engine exhaust fumes for coloring grapefruit in Florida. DENNY (5) after analysis of kerosene stove fumes concluded that the ethylene fraction was mainly responsible for the coloring of fruit and stated that ethylene when used in concentrations as low as 1 p.p.m. brings about this coloring. He also pointed out that a wide range of ethylene concentrations from 1 part in 2 million to 1 part in 5000 may be used to color fruit.

MACK (14), working on the blanching of celery with ethylene, found that 20 p.p.m. to 40 p.p.m. gave the optimum concentration for blanching and concentrations more than 40 p.p.m. gave less blanching.

It has been shown that a number of different fruits give off ethylene gas during ripening. While ELMER (6) appears to be the first to point out

that apples give off some form of physiologically effective gas, it remained
for GANE (7) to prove that the gas was ethylene. Other fruits have also
been found to give off ethylene. NIEDERL *et al.* (16) found that bananas
give off small amounts of ethylene while ripening. HANSEN and HARTMAN
(10) in 1935 gave considerable evidence that ethylene was given off by pears.
HANSEN (9), again working on pears and measuring the ethylene by chemi-
cal means, studied the relation of ethylene production to respiration. He
found that the amount of ethylene given off by the ripening of pears was
influenced greatly by such factors as temperature, anaerobic conditions, and
varietal differences. BIALE (2) and MILLER *et al.* (15) have shown that
emanations, believed to be ethylene, are given off by citrus fruit decaying
from the common green mold, *Penicillium digitatum* Sacc. MILLER *et al.*
(15) also found that sound citrus fruit gave slight but positive tests for
ethylene.

Without having some delicate simple test, it is impossible to know when
storage rooms are properly ventilated or whether the air being taken into
the storage rooms is free from ethylene. It has been our purpose, therefore,
to develop a method which could be used to test automobile exhaust gases
for ethylene and to detect small amounts of this gas in fruit storage rooms.

Method

The epinasty test using pea plants, with some modification of the method
as described by KNIGHT *et al.* (12) and KNIGHT and CROCKER (11), seemed
to offer the best possibility as a method of quantitatively measuring very
small ethylene concentrations.

Results

MEASUREMENT OF THE ETHYLENE EQUIVALENT OF MOTOR EXHAUST FUMES

Alaska peas were planted in cans 2½ inches high and 4 inches in diameter,
the cans being filled with one part sand, two parts water and three parts peat
moss. The peas were grown at 75° F. until the plants were one or two inches
high. This usually required four days.

One can of peas was placed in each of eight removable lid steel drums of
55-gallon capacity. After the peas were placed, the lids were clamped
tightly and different measured quantities of motor exhaust fumes were
placed in five of the drums; measured quantities of ethylene were placed
in two drums, and one was kept as a control. After four days, peas grown
in the exhaust fumes were compared with the others grown in the drums
with known quantities of ethylene and with the control to which no gas had
been added.

The automobile exhaust gases to be tested were first introduced into an
ordinary steel drum which served only as a reservoir. This was done by
means of a 1¾-in. hose one end of which was placed on the exhaust of a
Ford V8 automobile and the other end into the side opening of the drum.
The top of this drum contained a four-inch opening from which the gases

could escape.   The automobile motor was started and the gas fumes allowed
to run into the drum for about 15 minutes to displace the air.   The rate of
flow of the gas from the motor was measured by having an anemometer on
the opening in the top of the drum and the quantity of exhaust gases coming
from the motor in a given time was recorded.   A single barrel tire pump
had been made into a syringe by removing the valves, placing a double
leather on the plunger and calibrating it.   The pump was used to remove
given quantities of the motor gas from this drum and place it in the drums
containing peas.

A known amount of ethylene was introduced into two of the drums as
follows.   The ethylene was first introduced into a 5-gallon carboy to give a

FIG. 1.   Equipment used for measuring out small quantities of ethylene into epinasty
test drums.

concentration of 1 part ethylene to 2000 parts air mixture.   This was done
by means of water displacement from a burette having a 0.5-mm. capillary
tube connected at the top and a leveling bottle at the bottom.   The leveling
bottle was raised to fill the burette and capillary with water.   Then the
capillary was attached to the ethylene container and the water in the capil-
lary and most of that in the burette were displaced with ethylene.   The
capillary was then connected to the carboy and the desired amount of
ethylene introduced by raising the leveling bottle (see fig. 1B).   The carboy
also contained a piece of crumpled paper which helped to mix the gas con-
tents when the carboy was shaken.   The desired amount of this diluted gas
was then introduced into the barrels containing the pea plants by connecting
a closed graduated cylinder with a funnel and a capillary tube.   The capil-
lary tube was inserted into the bung hole of the drum.   By adding a given

quantity of water to the graduated cylinder air was forced into the carboy, displacing an equal amount of the gas mixture from the carboy into the drum with the peas. Direct water displacement was avoided since ethylene is appreciably water-soluble (see fig. 1A).

The ethylene and the concentrations obtained in the drums are given in table I.

TABLE I

DRUM NUMBER	GAS ADDED	AMOUNT OF GAS ADDED	CONCENTRATION IN DRUM
		ml.	*p.p.m.*
1	None		
2	Ethylene	0.05	0.25
3	Ethylene	0.1	0.5
4	Motor exhaust	25.0	120.0
5	Motor exhaust	80.0	400.0
6	Motor exhaust	160.0	800.0
7	Motor exhaust	400.0	2000.0
8	Motor exhaust	800.0	4000.0

Figure 2 shows a set of peas after four days in these drums. There was no bending due to epinasty in the control drum although the plants were tall and etiolated so that when removed for photographing several of them bent over from their own weight.

The peas in drum no. 2 with 0.25 p.p.m. of ethylene bent over and grew 2″ to 2½ in. horizontally. Those in no. 3 with 0.5 p.p.m. ethylene bent over and grew 1½ to 2 in. horizontally. Those in no. 4 with 120 p.p.m. of motor gas showed almost as much epinasty as those in 0.25 p.p.m. of ethylene. The
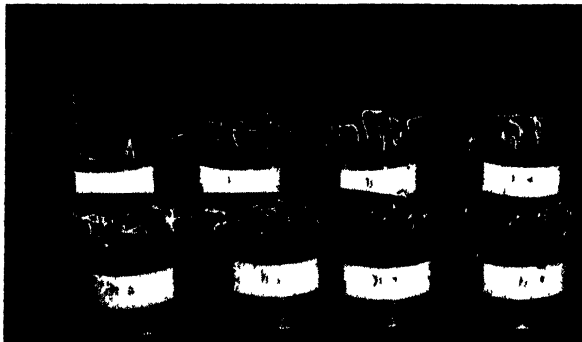
FIG. 2. Epinasty produced by ethylene and motor exhaust gases on Alaska peas.

    No. 1. Control (several peas bent over by their own
                weight after removing from test drum).
    No. 2. 0.25 p.p.m. ethylene
    No. 3. 0.5   p.p.m. ethylene
    No. 4.   118 p.p.m. motor exhaust
    No. 5.   400 p.p.m. motor exhaust
    No. 6.   800 p.p.m. motor exhaust
    No. 7. 2000 p.p.m. motor exhaust
    No. 8. 4000 p.p.m. motor exhaust

peas in drums nos. 5, 6, 7, and 8 showed progressively less plant growth. No. 8 showed almost no growth at all. The last four showed considerable swelling or enlargement of the stems.

From these results it appears that no. 4, with 120 p.p.m. of motor exhaust gas, was affected slightly less than no. 2 with 0.25 p.p.m. of ethylene. No. 5, with 400 p.p.m. of exhaust gas, was affected much more than no. 3 with 0.5 p.p.m. of ethylene. It would appear that drum no. 5 has about twice as high a concentration of ethylene as no. 3.

If we consider that no. 4 with 25 ml. of motor exhaust gas is the equivalent of no. 2 with 0.05 ml. of ethylene and that no. 5 has twice the ethylene equivalent of no. 3 we can calculate the ethylene equivalent of the motor fumes as follows:

  0.05 ml. ethylene = 25 ml. motor exhaust gas.
  1   ml. ethylene = 500 ml. motor exhaust gas.
  2000   ml. ethylene = 1,000,000 ml. motor exhaust gas or 2000 p.p.m.

The peas in no. 4 did not bend as sharply, and they grew a little longer than those in no. 2, indicating that the ethylene equivalent in no. 4 was not quite as great as that of no. 2. The ethylene equivalent of the motor gas in barrel no. 4 was probably more nearly equal to 0.04 ml. of ethylene; and assuming that the ethylene quivalent of the exhaust gas in the air in barrel no. 4 was equal to 0.04 ml. ethylene, then the exhaust gas has an ethylene equivalent of 1600 p.p.m. instead of 2000 p.p.m. The ethylene equivalent of the motor gas is then in the range of 1600 to 2000 p.p.m.

It was found that the car gave off 529 liters of exhaust gas per minute when idling. If this gas has an ethylene equivalent of 1800 p.p.m., then $\dfrac{529,000 \text{ ml.}}{1,000,000} \times 100 = 952$ ml. per minute of ethylene equivalent are given off by the car.

RELATION OF EPINASTY TO LEMON COLORING

Another experiment was set up in which 12 green lemons were placed in each of the 8 drums. The fruit for this test was selected as carefully as possible to get the same color, size, and type of lemons. The same amounts of ethylene and motor exhaust fumes were used as for the peas in the previous experiment. The lemons were left 10 days at 76° F. and then the drums were opened and the lemons examined for color.

The control fruit in no. 1 had colored very little.

The fruit in no. 2 was noticeably more colored than the control.

The fruit in no. 3 was not noticeably different from no. 2.

The fruit in no. 4 was slightly less colored than that in nos. 2 and 3.

The fruit in nos. 5, 6, 7, and 8 were progressively more colored, no. 8 almost fully colored.

These results indicate that the exhaust gases and ethylene are as effective in coloring lemons as they are in producing epinasty.

Another set of tests was run using different concentrations of ethylene. These data are given in table II.

TABLE II

DRUM NO.	ETHYLENE CONCENTRATION	p.p.m.	EFFECT AFTER 14 DAYS
1 Control	None		Slight change in color
2	1 part to 40 million	0.025	No noticeable difference from control
3	1 part to 20 million	0.05	A little more color than control
4	1 part to 10 million	0.1	Slightly more color than in no. 3
5	1 part to 5 million	0.2	Quite well colored
6	1 part to 2.5 million	0.4	Almost fully colored
7	1 part to 1 million	1.0	Almost fully colored
8	1 part to 0.5 million	2.0	Almost fully colored

These data cover a range of concentrations much lower than those used previously but the fruit were left in for a longer time. There was no noticeable effect in 14 days by concentrations of less than 1 part in 20 million.

Figure 3 shows the effect of a similar range of concentrations of ethylene
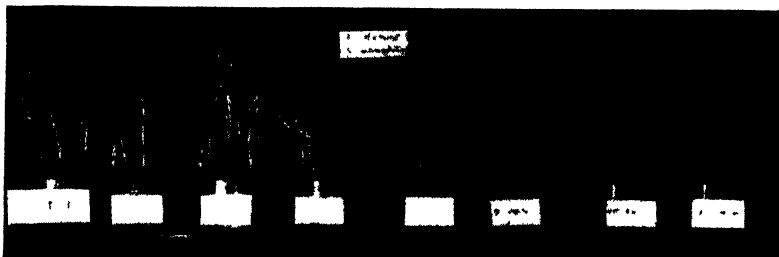
Fig. 3. Epinasty produced by different concentrations of ethylene on Alaska pea seedlings.

on peas under the same conditions as those used on the fruit. Table II and figure 3 show that there is a very close correlation between the concentrations necessary to cause bending in peas and coloring of lemons. Any ethylene concentration which is strong enough to cause epinasty or bending of the peas will cause an appreciable increase in the rate of coloring and maturing of lemons.

ETHYLENE EQUIVALENT CONTENT OF LEMON STORAGE ROOMS

More or less green mold decay always develops among lemons during the storage season. We questioned whether the ethylene given off by this decay might be an important factor in the premature coloring and maturing of the fruit; also whether the exhaust gases given off by cars and trucks and entering the storage rooms through fresh air intakes might have that effect.

Since the storage rooms are kept at a temperature of 55–60° F. it was necessary in order to run the epinasty test to devise some means of maintaining the peas in the storage room atmosphere and at the same time to keep them dark and at a temperature of about 76° F. These requirements were met by making a container consisting of a can 6″ in diameter and 11″ high with a maze effect at the bottom and in the cover so that the air could

come in around the bottom and go out at the top.   This can was then equipped with a heater consisting of 2 10-watt intermediate base electric lights connected in series.   Each light was covered with a collapsible metal tube to keep all light from the pea plants.   This heater was placed in the bottom of the container and covered first by a piece of asbestos then by a small metal stand.   On the stand was placed a can of peas previously grown in the can of peat moss and sand until 1 to 2 in. tall.   The heater (shown in fig. 4) maintains the peas at very nearly an even temperature as long as the room temperature and air movement are the same.   Different size lamps may be used in storages operated at other temperatures or thermostats may be employed.

This method of testing storage room air for the presence of ethylene appears to be quite satisfactory as a commercial method and is more accurate

Fig. 4.   Electrically heated dark chamber for growing peas in storage rooms.

than any of the usual chemical test methods.   Peas can be grown continuously in the storage room; when one set is removed others are put in their place.   Figure 5 shows pictures of peas grown in different lemon rooms.

Since figure 3 shows the effects of different known concentrations of ethylene, it can be used as a guide in estimating the concentration of ethylene in storage rooms.

The ethylene concentration of storage rooms may vary a great deal, depending on the amount of fresh air being brought in; the amount of fresh air brought in depending to a large extent upon the temperature and humidity outside as well as the refrigerating capacity available.

ETHYLENE EQUIVALENT PRODUCED BY DECAYING LEMONS

The rate of production of ethylene from one decaying lemon was roughly estimated by running air at the rate of 20 liters per hour through a jar containing a lemon decaying with *Penicillium digitatum* then through another jar containing a can of the pea seedlings.   The effect on the peas indicates a concentration of ethylene of about 1 part to 7 or 8 million parts of air.

This means that enough ethylene or its equivalent is added to 20 liters of air flowing through the jars per hour to equal a concentration of 1 part ethylene in about 7.5 million parts of air or $\dfrac{1}{7,500,000} \times 20$ liters per hour or $\dfrac{480}{7,500,000}$ liters per day.   This is equal to 0.064 ml. of ethylene per day or
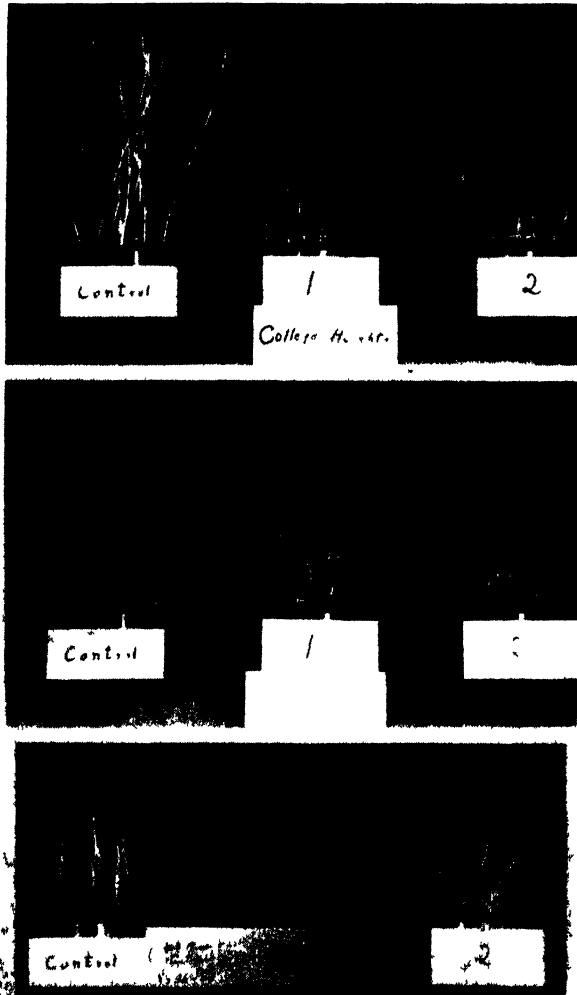
Fɪɢ. 5.   Epinasty effects obtained on pea seedlings by ethylene in three different lemon storage rooms.

about 1 ml. in 15 days.   While it has not been determined over how long a time this continues, it is considered probable that not much ethylene is given off after 15 days as the fruit is quite well decayed by that time.

Significance of Ethylene in a Storage Room

BɪALE and SHEPHERD (3) have shown that lemons give off CO_2 at the rate

of 7 to 8 mg. per kilo of fruit per hour at 58° F. or an average of 180 mg. of CO_2 per kilo of fruit per day.

The work of GREEN et al. (8) shows that the heat given off by fruit and vegetables is in direct proportion to the CO_2 given off. They also found that within the limits of experimental error this relationship is the same as that for the combustion of sugar. The heat of combustion of sugar is 673,000 calories per mole, and since 1 mole of sugar gives off 6 moles of CO_2, one mg. of CO_2 is equal to 2.54 calories.

If, as BIALE has shown, a kilogram of lemons give off 180 mg. of CO_2 per day, then they also give off 180×2.54 calories or 457 calories of heat. One pound of lemons would give off 207 calories per day.

One car of lemons in storage contains 655 boxes of 50 lb. each or about 32,750 lb. Then 32,750 lb. $\times 207$ or 6,779,250 calories of heat are given off per car per day at 59° F. Since 6,779,250 calories is the heat required to melt 187 lb. of ice, every 10.7 cars of stored fruit will require one ton of ice every 24 hours to remove the heat given off by the normal respiration.

BIALE and SHEPHERD (3) have shown that the respiration rate of normal lemons is approximately doubled by the respiratory gases given off by one lemon decaying with *Penicillium digitatum* when these gases are mixed with air and passed over the fruit at the rate of 3.6 liters per minute, a rate more than adequate to supply the oxygen for respiration of the fruit involved.

If it is assumed that this approaches a condition equal to that obtained for 1 per cent. green mold decay in a lemon storage room, then one additional ton of ice is going to be required by each 10.7 cars of lemons stored under these conditions.

Discussion

While the epinasty test has been known to be an indicator of the presence of ethylene, to the author's knowledge it has not been used to any extent commercially. Records of the California Fruit Growers Exchange indicate that the idea of using epinasty of tomatoes to measure ethylene in lemon storages existed as long as ten years ago. No way of growing tomato plants successfully in the low temperatures of the lemon storage was available at that time. In the present investigations it has served admirably as an accurate and simple test for ethylene.

One of the questions which will no doubt arise in the reader's mind is whether the carbon monoxide or some other of the automobile gases may not be responsible for the epinasty observed in experiments with these gases. CROCKER, ZIMMERMAN and HITCHCOCK (4) tested 38 different gases but found only five which produced epinastic effects and the minimum effective concentrations of those gases were as follows:

Ethylene	0.2 p.p.m. in air
Acetylene	250.0 p.p.m. in air
Propylene	1000.0 p.p.m. in air
Carbon monoxide	5000.0 p.p.m. in air

Butylene is not listed as affecting peas but is given as $\dfrac{1}{500,000}$ as effective as ethylene on tomatoes.

If there were as much acetylene as ethylene in the automobile exhaust gases it would be responsible for only $\dfrac{1}{1250}$ or about one-twelfth of one per cent. of the effect. KOBER and HAYHURST (13) list motor exhaust gases as containing about 9.3 per cent. carbon monoxide. This would be about 93,000 p.p.m. or about 52 times as much as ethylene; but since ethylene is 25,000 times as effective in producing epinasty, only about 0.2 of one per cent. of the effect would be due to carbon monoxide. It therefore becomes evident that carbon monoxide is of little consequence as compared to the ethylene in the automobile fumes.

The epinasty test for ethylene content in commercial storage rooms reported here is very simple and easily cared for after it is set up. It requires no technical skill except as is required to estimate the extent of epinasty in the pea plants in storage as compared with those kept as controls. In some locations in urban or other heavy traffic areas it may be difficult at times to find places to keep the control plants where they are not affected.

Two or three hours a week of a man's time is sufficient to keep tests going under most storage conditions.

Summary

1. This paper describes methods of using the epinasty reaction of pea seedlings for measuring small concentrations of ethylene in the exhaust fumes of automobile and in fruit storage rooms.

2. Data indicate a close relationship between the quantity of ethylene necessary to color lemons and that necessary to cause epinasty to pea seedlings.

3. The concentration of ethylene necessary to cause noticeable epinasty or lemon coloring has been found to be between 0.025 p.p.m. (1 part ethylene in 40 million) and 0.05 p.p.m. (1 part in 20 million) of air.

CALIFORNIA FRUIT GROWERS EXCHANGE
ONTARIO, CALIFORNIA

LITERATURE CITED

1. BARGER, W. R., and HAWKINS, LON A. Coloring citrus fruits in Florida. U.S.D.A. Bull. 1367. 1926.
2. BIALE, J. B. Effect of emanations from several species of fungi on respiration and color development of citrus fruits. Science n. s. **91**: 458–459. 1940.
3. BIALE, J. B., and SHEPHERD, A. D. Respiration of citrus fruits in relation to metabolism of fungi. Amer. Jour. Bot. **28**: 263–270. 1941.
4. CROCKER, WM., ZIMMERMAN, P. W., and HITCHCOCK, A. E. Ethylene-induced epinasty of leaves and the relation of gravity to it. Contrib. Boyce Thompson Inst. **4**: 177–218. 1932.

5. DENNY, FRANK E. Method of coloring citrus fruits. U. S. patent 1,475,938, December 4, 1923.

6. ELMER, O. H. Growth inhibition of potato sprouts by the volatile products of apples. Science n. s. **75**: 193. 1932.

7. GANE, R. The formation of ethylene by plant tissues and its significance in the ripening of fruits. Jour. Pomol. Hort. Sci. **13**: 351–358. 1935.

8. GREEN, W. P., HUKILL, W. V., and ROSE, D. H. Calorimetric measurements of the heat of respiration of fruits and vegetables. U.S.D.A. Techn. Bull. 771. 1941.

9. HANSEN, ELMER. Quantitative study of ethylene production in relation to respiration of pears. Bot. Gaz. **103**: 543–558. 1942.

10. HANSEN, ELMER, and HARTMAN, HENRY. The occurrence in pears of metabolic gases other than carbon dioxide. Oregon Agr. Exp. Sta. Bull. 342. 1935.

11. KNIGHT, LEE I., and CROCKER, WM. Toxicity of smoke. Bot. Gaz. **55**: 337–371. 1913.

12. ————, ROSE, R. C., and CROCKER, WM. Effect of various gases and vapors upon etiolated seedlings of sweet peas. Science n. s. **31**: 635–636. 1910.

13. KOBER, G. M., and HAYHURST, E. R. Industrial health. P. Blakiston's Son & Company, Philadelphia, p. 389. 1924.

14. MACK, W. B. The action of ethylene in accelerating the blanching of celery. Plant Physiol. **2**: 103. 1927.

15. MILLER, E. V., WINSTON, J. R., and FISHER, D. F. Production of epinasty by emanations from normal and decaying citrus fruits and from *Penicillium digitatum*. Jour. Agr. Res. **60**: 269–278. 1940.

16. NIEDERL, J. B., BRENNER, M. W., and KELLEY, J. N. The identification and estimation of ethylene in the volatile products of ripening bananas. Amer. Jour. Bot. **25**: 357–361. 1938.

17. SIEVERS, A. F., and TRUE, R. H. A preliminary study of the forced curing of lemons as practiced in California. U.S.D.A. Bur. Pl. Ind., Bull. 232. 1912.

18. WRIGHT, R. C. Coloring satsuma oranges in Alabama. U.S.D.A. Bull. 1159. 1923.

RESPIRATION OF MOSAIC–INFECTED TOBACCO PLANTS

F . L Y L E W Y N D

(WITH ONE FIGURE)

There has been reported in the literature a number of attempts to observe *in vitro* the respiration of virus preparations in an effort to detect direct evidence of a valid metabolic activity. EATON (10) studied manometrically the respiration of cultures of Staphylococcus lysed by bacteriophage and found a detectable amount of oxygen absorption and carbon dioxide production. On the other hand, BRONFENBRENNER (2, 3) BRONFENBRENNER and REICHERT (4), BACHMANN and WOHFIEL (1), WOHFIEL (19), PARKER and SMYTHE (15), and WYND and BRONFENBRENNER (21), using several types of extraordinarily sensitive procedures, were unable to observe any evidence of a respiratory metabolism by preparations of bacteriophage. PIRIE and HOLMES (16) reported that Agalactia virus absorbed large amounts of oxygen when suspended in a saline solution containing lactate, and these authors believed, therefore, that virus particles were living organisms.

The respiratory rates of green plants infected with virus have also received a considerable amount of study. BUNZEL (5) remarked that sugar beets stunted by the Curly-top disease suffered from a "fever." Later (6) he stated that a respiratory study of these "feverish" plants would be highly desirable in the effort to interpret the more exact nature of the disturbances which the disease produced. THUNO (18) reported that potatoes infected with leaf-roll eliminated more carbon dioxide than healthy tissue, although a study of his data shows that this was not always true. DUNLAP (9) studied the rates of carbon dioxide production of a number of species and found that the virus-affected plants exhibited an increased respiration in their younger tissues and a decreased rate in the older tissues. He observed the mosaic leaves of tobacco, tomato, pokeweed (*Phytolacca decandra*), cucumber, and raspberry, and yellows-affected leaves of peach, plum, aster, and ragweed (Ambrosia). The conclusions of DUNLAP (9) were criticized by CALDWELL (7) because the data were obtained from tissues respiring in an atmosphere of reduced oxygen content. Further, he doubted if efficient absorption of carbon dioxide was obtained under the condition of the experiment. He also believed that the period of the observation was too short. CALDWELL collected in barium hydroxide the carbon dioxide produced and titrated the remaining alkali after 48 hours. He found an increased carbon dioxide production by virus-infected tomato plants. LEMMON (14) made an extensive study of the comparative rates of metabolism of healthy and mosaic-infected tobacco leaves and found that the diseased leaves always had a lower respiratory rate than normal leaves. His data were expressed in terms of the fresh weight of excised discs of tissue. He further observed that discs of healthy tissue weighed only 85 per cent. as much as did the equal discs of diseased tissue.

The present work on the respiratory rates of healthy and mosaic-affected tobacco leaves was undertaken in an effort to explain the conflicting data on the respiration rates of virus-infected green plants, and to accumulate evidence on the nature of the substance responsible for the tobacco mosaic disease.

Procedure

Seeds of Burley tobacco were germinated in the greenhouse and transplanted into two-inch pots as soon as the plants were large anough to handle. As growth progressed they were transplanted into four-inch and finally into eight-inch pots. A great many more plants were grown than were needed for the experimental work in order that plants identical in appearance could be selected at the proper time. The plants were large enough for study by the first of May, nine weeks from the time of planting. When the plants had five well-developed leaves, the two lower leaves were removed. the lowest remaining leaf dsignated as number 1, and the higher leaves numbered consecutively. Several new leaves appeared during the course of the observations, and they were designated by consecutively higher numbers as soon as they reached a size large enough to be included in the experimental material.

A virus preparation was made by grinding the upper leaves of severely infected plants and squeezing the juice through cheesecloth. Inoculations were made by dipping a small pad of cheesecloth into this juice and gently rubbing the surface of the leaf. In all cases, only leaf 1 received the inoculation. At intervals of two or three days, three control plants and three inoculated plants were removed to the laboratory for study.

The rate of oxygen use was determined on a composite sample obtained by cutting three discs from each leaf, equally numbered from each of the three plants. The data therefore represent the total oxygen use of nine discs, three from a leaf of comparable age from each of three plants. The discs were cut by a sharp cork borer ten millimeters in diameter. Their oxygen consumption was determined at 25° C. by the Barcroft differential manometers. Since the data of LEMMON show that healthy discs weighed only 85 per cent. as much as those of diseased tissue, it is seen that the present procedure of comparing tissue samples having an equal area gives differences varying somewhat in magnitude from those which would have been obtained if equal weights had been used. Both of these methods of comparison are subject to error since neither the weight nor the area of tissue samples strictly represents the absolute amount of living protoplasm.

After the discs were removed from the leaf, the remaining tissue was finely ground in a mortar with purified quartz sand and the juice extreated by squeezing the pulp through several thicknesses of cheesecloth. Tests for the presence of the virus were made by the inoculation of young tobacco plants with this juice. HOLMES (11) has correctly pointed out that the inoculation of the press juice detects only an *infectious* concentration of

the virus, and not necessarily its first appearance. Determinations of reducing sugar, invertase, oxygenase, peroxidase, and catalase were also made on each of these juice samples and these data have been reported in a previous paper (20).

Experimental data

Examination of the data presented in table I shows the time necessary for the appearance of infectivity in various leaves of the plant after the inoculation of the lower leaf (number 1). Leaf 1 was infectious from the beginning of the experiment, as could be expected, since it received the inoculation. The virus left on the surface from the inoculation itself could have caused infection in this instance. Leaves 2 and 3 became infectious on the fourteenth day, leaf 4 on the eighth day. Leaves 5 and 6 were not yet present when the inoculation occurred, yet they appear to be among the

TABLE I

THE TIME NECESSARY FOR THE APPEARANCE OF VIRUS IN VARIOUS LEAVES OF TOBACCO PLANTS AFTER INOCULATION OF LEAF 1 (THE LOWEST) WITH VIRUS +, VIRUS PRESENT; —, VIRUS ABSENT; 0, LEAF NOT YET PRESENT

DAYS AFTER INOCULATION	LEAF 1	LEAF 2	LEAF 3	LEAF 4	LEAF 5	LEAF 6
2	+	−	−	−	0	0
4	+	−	−	−	0	0
6	+	⊦*	−	−	+	0
8	+	−	−	+	−	−
11	+	−	−	+	+	−
14	+	+	+	+	−	+
16	+	+	+	+	−	+
18	+	+	+	+	+	+
21	+	+	+	+	−	+

* This appears to have been due to the use of an infected plant since 8 days elapsed before the second leaf became infectious in all other experiments.

first tissues to become infectious. It seems probable that the upper tissues were the first to become infectious by means of newly formed virus material, even though the lowest leaf had received the inoculation. Under the growing conditions of the present experiments, the entire plant became infectious at about the fourteenth day. The duration of this period would vary with the growing conditions, and with the age and vitality of the plant.

The cubic millimeters of oxygen used by leaves from normal and from inoculated plants are presented in table II. These rates of oxygen use by leaves from inoculated plants, calculated as the percentage of the use by leaves of similar age from normal plants, are presented in table III. It is difficult to obtain exactly equal samples of leaf tissue by the method described above. For instance, younger leaves differing only slightly in their stage of development may vary greatly in the extent of their rolling and evenness of their surfaces, and since equal areas are used as the basis for

TABLE II

THE USE OF OXYGEN IN CUBIC MILLIMETERS BY NINE EXCISED DISCS FROM LEAVES OF NORMAL AND INOCULATED TOBACCO PLANTS. N, LEAVES FROM NORMAL PLANTS; IN, LEAVES FROM INOCULATED PLANTS

SECOND DAY

TIME	LEAF 1 N	LEAF 1 IN	LEAF 2 N	LEAF 2 IN	LEAF 3 N	LEAF 3 IN
min.	cu. mm.	cu. mm.	cu. mm.	cu. mm.	cu. mm.	cu. mm.
15	11.0	10.9	8.7	11.1	13.9	12.9
30	22.2	23.4	17.7	22.0	27.6	26.8
45	33.0	34.3	27.4	33.2	40.5	40.0
60	44.4	45.5	36.8	44.0	54.2	53.0

FOURTH DAY

TIME	LEAF 1 N	LEAF 1 IN	LEAF 2 N	LEAF 2 IN	LEAF 3 N	LEAF 3 IN
min.	cu. mm.	cu. mm.	cu. mm.	cu. mm.	cu. mm.	cu. mm.
15	16.1	21.4		19.5	15.0	18.1
30	27.4	37.6		33.6	28.0	32.6
45	36.2	49.6		46.0	40.8	45.5

SIXTH DAY

TIME	LEAF 1 N	LEAF 1 IN	LEAF 2 N	LEAF 2 IN	LEAF 3 N	LEAF 3 IN
15	6.9	8.6	8.1	9.3	14.4	13.8
30	14.8	17.5	16.4	18.6	28.6	24.7
45	22.2	26.7	25.0	28.4	43.0	37.5
60	30.6	36.0	34.2	37.8	57.6	49.8

EIGHTH DAY

TIME	LEAF 1 N	LEAF 1 IN	LEAF 2 N	LEAF 2 IN	LEAF 3 N	LEAF 3 IN
15	8.8	8.8	8.8	7.4	12.2	11.2
30	17.0	15.9	16.7	14.4	24.5	22.4
45	25.2	23.8	25.0	22.1	36.8	34.7
60	33.9	31.0	33.4	29.5	49.5	47.0

ELEVENTH DAY

TIME	LEAF 1 N	LEAF 1 IN	LEAF 2 N	LEAF 2 IN	LEAF 6 N	LEAF 6 IN
15	7.2	8.4	9.5	7.9	11.8	10.7
30	15.0	16.2	16.7	15.5	22.3	21.5
45	25.8	24.2	25.2	23.5	33.1	32.2
60	30.6	31.9	31.2	30.7	43.3	42.2

FOURTEENTH DAY

TIME	LEAF 1 N	LEAF 1 IN	LEAF 2 N	LEAF 2 IN	LEAF 6 N	LEAF 6 IN
15	7.3	9.6	10.2	8.6	12.9	11.5
30	15.7	18.4	21.9	17.2	27.0	23.2
45	25.2	27.0	30.6	26.8	40.7	35.8
60	34.1	35.4	39.4	35.8	53.9	48.8

SIXTEENTH DAY

TIME	LEAF 1 N	LEAF 1 IN	LEAF 2 N	LEAF 2 IN	LEAF 6 N	LEAF 6 IN
15	10.5	10.9	11.6	10.6	13.4	6.0
30	20.5	21.5	21.8	20.4	26.8	13.2
45	29.7	31.7	31.0	30.7	39.3	20.0
60	39.5	41.3	39.8	41.8	51.8	30.2

EIGHTEENTH DAY

TIME	LEAF 1 N	LEAF 1 IN	LEAF 2 N	LEAF 2 IN	LEAF 6 N	LEAF 6 IN
15	8.8	6.1	9.1	6.9	9.3	9.4
30	17.6	13.8	16.3	14.0	19.2	19.1
45	27.1	22.1	25.2	22.0	28.6	28.8
60	35.6	29.8	35.6	29.3	38.4	38.3

TWENTY-FIRST DAY

TIME	LEAF 1 N	LEAF 1 IN	LEAF 2 N	LEAF 2 IN	LEAF 3 N	LEAF 3 IN
15	8.6	8.4	8.8	7.7	10.3	7.4
30	16.6	17.1	16.9	15.6	20.1	15.2
45	25.2	25.8	24.6	22.7	29.4	22.6
60	33.4	33.7	32.5	30.4	37.8	29.4

TABLE III

THE USE OF OXYGEN DURING THE TIME INDICATED BY LEAVES FROM INOCULATED TOBACCO
PLANTS CALCULATED AS PERCENTAGE OF THE OXYGEN USE BY LEAVES
FROM THE NORMAL PLANTS

DAYS AFTER INOCU-LATION	OXYGEN USE IN 15 MINUTES				OXYGEN USE IN 30 MINUTES			
	LEAF 1	LEAF 2	LEAF 3	LEAF 6	LEAF 1	LEAF 2	LEAF 3	LEAF 6
	%	%	%	%	%	%	%	%
2	99.3	127.6	92.7	....	105.3	125.7	97.3	... ...
4	132.9	....	120.6	....	137.5	....	116.5	....
6	124.6	113.0	96.0	...·.....	118.3	113.5	86.4	...
8	100.0	84.4	91.7	....	93.5	86.3	91.3	....
11	116.7	83.3	....	90.8	107.9	92.8	....	96.5
14	131.8	76.8	....	89.3	117.8	78.7	....	85.7
16	103.9	91.5	... .	44.8	105.0	93.8	... .	49.3
18	69.3	75.9	....	101.3	78.4	86.0	... .	99.7
21	97.7	87.5	....	71.9	103.0	92.5	....	75.6

DAYS AFTER INOCU-LATION	OXYGEN USE IN 45 MINUTES				OXYGEN USE IN 60 MINUTES			
2	103.8	121.4	99.0	....	102.5	119.8	97.9	... .
4	137.7	....	116.5	....	....	... .	.... .	.... .
6	121.5	113.8	87.3	....	117.6	110.5	86.4	.. ...
8	94.3	88.5	94.4	....	91.4	88.3	94.9	... ...
11	93.5	93.4	... .	97.3	104.2	98.4	..	97.4
14	107.5	87.5	....	87.9	103.8	90.8	... .	90.5
16	106.5	99.2	....	50.9	104.5	105.0	......	58.3
18	81.7	87.5	... .	100.7	83.7	82.3	....	99.7
21	102.6	92.3	....	.77.0	100.9	93.5	.. . ...	77.7

comparison, erroneous results are sometimes obtained. Therefore, the data are presented in considerable detail, so that the reader may judge the validity of the conclusions presented below.

A survey of the data shows the following relationships to exist. At the time the experiment was begun, there were three leaves large enough for study. On the second day after inoculation of leaf 1 (the lower), none of the leaves showed any significant change in the rate of oxygen use as compared to the rates of leaves from normal plants. An exception in the case of leaf 2 is probably an error because the observed rate of leaf 2 from normal plants used for comparison was too low. From table II, it is seen that leaf 2 of normal plants has a value of only 8.7, although it is obvious that it should have been about 11.0. This is a reasonable assumption because the data presented in the present paper and certain other data obtained in the writer's laboratory indicate that the rate of oxygen use increases progressively in leaves from the upper portion of the tobacco plant.

On the fourth day after inoculation, all three leaves showed an abrupt increase in oxygen use. From figure 1 it is seen that leaf 1 showed the greatest increase, and that this increase was progressively less in the upper leaves. On the sixth day, this increase was yet apparent, although it had diminished in magnitude. By the eighth day, however, the leaves from the inoculated plants were either less than those of normal plants, or were ap-

proaching the control value. In view of subsequent determinations, presented in table I, it is possible that these values are too low. However, the fact that each determination was made on a composite sample from three plants indicated that the lessened rate was approximately that of its observed value. After the eleventh day, the respiration rates became progressively less, although these rates of diminution became less rapid with time. This lessened rate could not have been due to a mere advance in maturity since leaf 6 also had a lessened rate even though it was the youngest leaf.

Six manometers were available which permitted the comparison of only three sets of leaves. By the eleventh day, two additional leaves, numbered 5 and 6 respectively, were large enough for study, and since the terminal
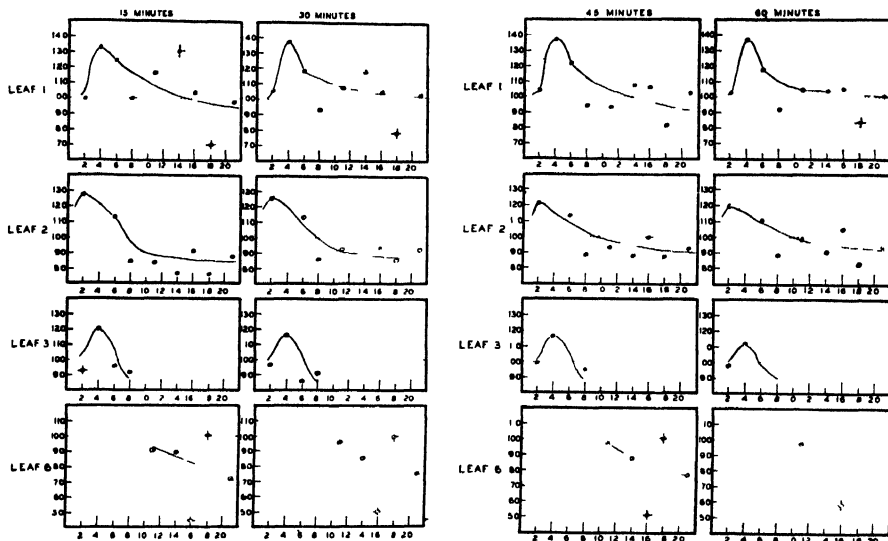

Fig. 1. The oxygen consumption of 9 discs of tissue excised from the leaves of tobacco plants inoculated with mosaic virus. The ordinates represent the data in terms of percentage of the oxygen use by discs from normal leaves of similar age. The abscissae represent the days elapsed since inoculation.

part of the plant is apparently especially concerned with the production of virus, the observations on the originally terminal leaf 3 were discontinued in favor of leaf 6. Even before leaf 6 appeared, infected plants exhibited a generally lessened respiration rate and this characteristic occurs from the very beginning in the newly formed leaves. It is interesting to note that the rates of oxygen use by leaf 6 appear to fall on the latter part of the curve typical for the older leaves which were on the plant from the time of inoculation.

A comparison of respiratory rates presented in figure 1 with the data in table I shows that the times necessary for the appearance of infectivity in the various leaves are not at all related to the periods of disturbed metabolism indicated by the increased rate of oxygen use. The peak of the disturbed oxygen use occurred at about the fourth day *for all leaves*. At this

time, leaf 1 contained the virus, since it had received the inoculation, but the upper leaves had not yet developed the power to infect young normal plants. Unfortunately, the lack of equipment prevented a study of the oxygen consumption in all leaves, and consequently, the rates of leaves 4 and 5 are unknown.

The curves showing the amount of oxygen used in 60 minutes are probably the most authentic since the magnitude of the manometer reading was greater. This set of figures shows that the inoculated lower leaf regained an approximately normal rate of respiration after the eleventh day, even though it contained the active virus. Leaf 2, at about the same time, had assumed a rate less than normal. The study of leaf 3 was discontinued on the eighth day in favor of leaf 6, and this later leaf assumed a rate of respiration lower than any other. The magnitude of this decrease in rate is progressively greater in the younger leaves.

Discussion

It has been shown by HOLMES (**11, 12, 13**) and SAMUEL (**17**) and by the review by CRAFTS (**8**) that the virus spreads from the point of infection by a comparatively slow movement from cell to cell. After three or four days, the phloem is invaded and then in a remarkably short time the virus reaches other parts of the plant. It is this period of dispersal through the phloem tissue that seems to coincide with the sudden disturbance of the respiratory metabolism.

This disturbance preceded by about ten days the appearance of the virus in infectious concentration throughout the plant. Although the initial disturbance in metabolism occurred simultaneously in all leaves, the newly formed virus attained infectious concentration first in the upper leaves. These also are the leaves having the greater metabolic activity.

If the physiological disturbances indicated by the change in the rates of oxygen use were manifestations of the activity of the virus particles *per se*, then these changes should accompany the increase in the number of these particles. On the other hand, if the mosaic disease is caused by some chemical product of abnormal metabolism, which like the bacteriophage, could itself initiate a series of reactions which would produce new virus material during some subsequent process, one might then expect to detect a disturbed metabolism before the appearance of any considerable amount of new virus substance. Although observations of the latter type do not demonstrate conclusively the validity of such an inference, they do indicate that the increased rate of respiration could not be due to the metabolism of the virus particles themselves.

It is apparent that the data on the respiration of mosaic infected plants published by DUNLAP and by LEMMON are not necessarily contradictory since diseased plants have either an increased or decreased rate depending on the time which has elapsed since infection.

Summary

1. The rate of oxygen use by leaves from mosaic-affected tobacco plants is greatly increased by the fourth day after a lower leaf was inoculated.

2. The period of stimulated oxygen use precedes by approximately ten days the general appearance of the mosaic agent in infectious concentration.

3. Although the disturbed metabolism indicated by the increased rate of oxygen use occurs simultaneously throughout the plant, the appearance of infectious concentration of new virus material occurs first in the upper leaves, excepting, of course, the leaf which had received the inoculation. These leaves have a higher initial rate of metabolism.

4. By the time infectious concentrations of virus appear in the upper leaves, the rates of oxygen use by these leaves are always less than that of normal leaves.

5. Since infectious material appears only subsequent to a disturbed metabolism, it is probable that the observed metabolic changes are cellular in nature and do not depend on any metabolic activity of the virus material.

LITERATURE CITED

1. BACHMANN, W., and WOHFIEL, T.   Stoffwechselversuche mit einen Coli-Bacteriophagen. Zentr. Bakt. Parasitenk., Abt. 1. Orig. **104**: 256–261. 1927.

2. BRONFENBRENNER, J. J.   Further studies on so-called bacteriophage. Proc. Soc. Exp. Biol. Med. **22**: 81–82. 1924.

3. ——————.   Does bacteriophage respire?   Science, n.s. **63**: 51–52. 1926.

4. ——————, and REICHERT, PHILIP.   Respiration of so-called filter-able viruses. Proc. Soc. Exp. Biol. Med. **24**: 176–177. 1926.

5. BUNZEL, HERBERT H.   A biochemical study of the curly-top of sugar beets.   U. S. Dept. Agr., Bur. Pl. Ind., Bull. 277. 1913.

6. ——————.   Oxidases in healthy and in curly-dwarf potatoes. Jour. Agr. Res. **2**: 373–404. 1914.

7. CALDWELL, JOHN.   The physiology of virus diseases in plants. VI. Some effects of mosaic on the metabolism of the tomato. Ann. Appl. Biol. **21**: 206–224. 1934.

8. CRAFTS, A. S.   Movement of viruses, auxins and chemical indicators in plants.   Bot. Rev. **5**: 471–504. 1939.

9. DUNLAP, ALBERT A.   The total nitrogen and carbohydrates, and the relative rates of respiration in virus infected plants. Amer. Jour. Bot. **17**: 348–357. 1930.

10. EATON, MONROE D. A quantitative study of the respiration of Staphylococcus cultures lysed by bacteriophage. Jour. Bact. **21**: 143–156. 1931.

11. HOLMES, FRANCIS O. Local and systematic increases of tobacco mosaic virus. Amer. Jour. Bot. **17**: 789–805. 1930.

12. ——————. Local lesions of mosaic in *Nicotiana tabacum* L. Contrib. Boyce-Thompson Inst. **3**: 163–172. 1931.

13. ——————. Symptoms of tobacco mosaic disease. Contrib. Boyce-Thompson Inst. **4**: 321–357. 1932.

14. LEMMON, PAUL. Comparative studies on metabolism of healthy and mosaic-infected tobacco leaves. Respiration studies. Amer. Jour. Bot. **22**: 912. 1935.

15. PARKER, R. F., and SMYTHE, C. V. Immunological and chemical investigation of vaccine virus. V. Metabolic studies of elementary bodies of vaccinia. Jour. Exp. Med. **65**: 109–120. 1937.

16. PIRIE, ANTOINETTE, and HOLMES, BARBARA ELIZABETH. A study of the enzymes of the Agalactia virus. British Jour. Exp. Path. **14**: 290–296. 1933.

17. SAMUEL, GEOFFREY. The movement of tobacco mosaic virus within the plant. Ann. Appl. Biol. **21**: 90–111. 1934.

18. THUNG, T. H. Physiologisch Onderzock met Betrekking tot het Virus der Bladrolziekte van de Aardappelplant, *Solanum tuberosum* L. Tijdschrift over Plantenziekten **34**: 1–74. 1928.

19. WOHFIEL, TRAUGOTT. Experimetelle Beiträge zur Theorie der bacteriophagen Lyse. Zeitschr. Hyg. Infektionskrankh. **108**: 733–745. 1928.

20. WYND, F. LYLE. Certain enzymatic activities of normal and mosaic-infected tobacco plants. Jour. Gen. Physiol. **25**: 649–661. 1942.

21. ——————, and BRONFENBRENNER, J. J. The nature of the catalase reaction in the residue of *Staphylococcus aureus* lysed by bacteriophage. Jour. Bact. **38**: 659–675. 1939.

RIDGES AND SECTORS INDUCED IN THE RIND OF CITRUS FRUITS BY FUMIGATION WTH HYDROCYANIC ACID[1]

WALTON B. SINCLAIR AND DAVID L. LINDGREN

(WITH FOUR FIGURES)

The fumigation of citrus trees with hydrocyanic acid (HCN) for pest control, at certain times of year and under certain environmental conditions, produces irregular and excessive growth of the outer peel (flavedo) of the fruit (3). This abnormality is commonly known as "ridging" or "coxcombing." That ridging occurs in some groves to a greater extent than in others has been generally recognized, but growers and field men have until recently been unable to trace the cause.

In some fruits, the growth may appear as a single smooth ridge in the form of a longitudinal sector; other fruits may have several longitudinal sectors of equal or unequal width (fig. 1). The amount of ridging varies considerably. The ridges may extend the full length of the fruit, from the calyx to the stylar end, or they may be small and irregularly placed over the entire fruit surface.

Deformations or variations on fruit surfaces occur naturally, to a limited degree, as bud variants in all varieties of citrus (2). SHAMEL and his co-workers (4) have studied these bud variations intensively from a commercial standpoint, identifying them largely by their appearance on the fruit. They have shown that individual fruit variations from limbs on given trees can be transmitted by means of bud propagation. They state: "The degree of inherent stability of the progenies has been found to be about the same as the condition of uniformity in the characteristics of the parent bud variations. If the fruit or foliage of these limb or tree variations was uniform their progenies have been found to be uniform; on the other hand, if there was a marked variability of the fruit or leaves in the parent limb variations the progenies have usually been relatively variable."

The purpose of this paper is to show that certain individual fruit variations, similar to those ordinarily transmitted and produced by means of bud propagation, can, under certain conditions, be induced by fumigation with HCN.

It was decided to determine the amount of ridging on the fruit of trees fumigated at different seasons of the year. Groves in which some trees could be fumigated at various times of year and others, to serve as controls, could go without fumigation, were selected for this investigation. As citrus is grown in southern California in both coastal and inland districts, experimental plots were located in commercial groves in both of these areas. For more than two years, extensive counts of the ridged fruits in the different groves were made. The results of the experiments, showing the

relation between the time of year of the fumigation and the amount of sectors and ridges formed on the fruits, are of sufficient interest and importance to warrant publication at this time.

Experimental procedure

In southern California, citrus trees are fumigated with HCN for pest control from mid-July to early spring, a period of approximately eight months in which commercial fumigators can operate. During the months of May, June, and part of July fumigation is discontinued as fruits are then more susceptible to injury than at other times of year. For a short period in the fall, also, usually in October, the trees are rather susceptible and fumigation is suspended. The data reported herein were obtained from
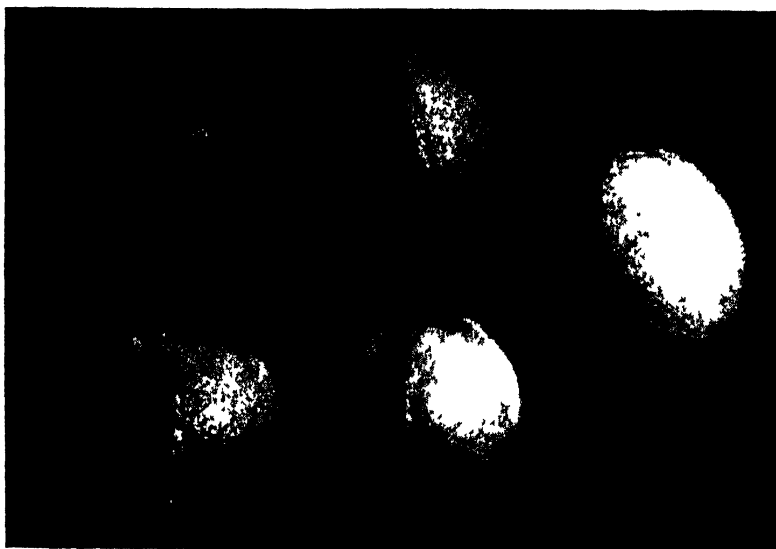

FIG. 1. Ridged Valencia oranges and a normal fruit from trees which had 31.4 per cent. of their fruits affected (Grove 2, table I).

trees fumigated in November, December, January, and February, preliminary experiments having indicated that trees fumigated during January and February were the ones on which ridging of the fruits was most likely to occur.

The experiments were so planned that the fumigated trees were scattered throughout the plots. (Figure 2 illustrates the type of plot design.) Fumigation of certain trees in this manner is commonly referred to in fumigation practice as "spot fumigation," in contrast with the usual method, in which all the trees of an orchard are fumigated. A distinct advantage in fumigating scattered trees is that untreated trees (controls) are in close proximity to adjacent treated trees and receive similar cultural treatment. This condition partially equalizes any possible effects on ridging due to variations in soil, fertilization, irrigation, etc. The concentration of HCN

used in each fumigation varied from 20 ml. to 28 ml. per unit. A unit in citrus fumigation is approximately 100 cu. ft. for the average-sized tree.

The amount of fruit that was ridged was determined by counting 200 fruits, at random, around each of the trees, on inside and outside positions, and recording the number affected. The percentages were calculated on a basis of 200 fruits per tree.
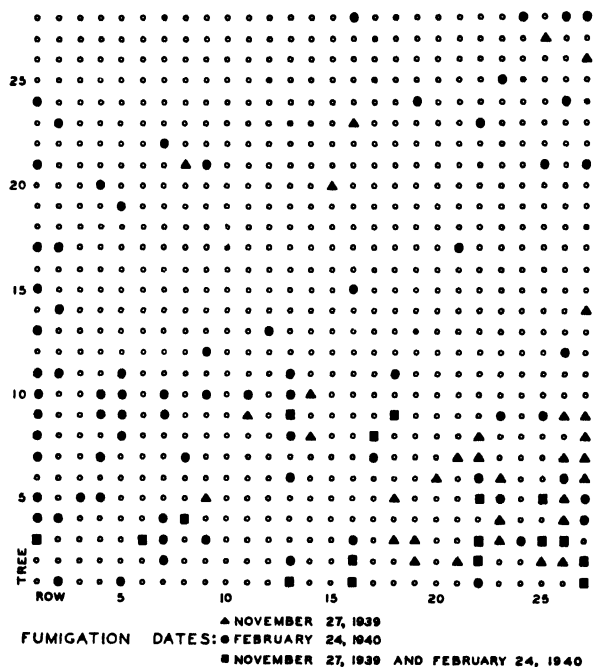

FIG. 2. Diagram of an experimental plot, showing location of trees fumigated at different times of year.

Results

The results shown in table I are typical of those obtained in this investigation. Data are reported for navel and Valencia oranges, grapefruit, and lemons and are so arranged that the average percentages of ridged fruits on trees receiving the different treatments in each grove can be compared. As each grove represents a different experiment in a different location, a brief description of the results in each grove is given.

Some very interesting comparisons can be made from the data reported from grove 1 (table I). Only a small percentage (0.1 per cent.) of the fruits counted on the unfumigated trees (controls) were ridged. Of fruits counted December 11, 1940, on 24 trees fumigated February 24, 1940, an average of 36 per cent. had ridges and sectors. Counts of fruits were also made December 11, 1940, on 18 trees that had been fumigated November 27, 1939. The average amount of ridged fruits on these trees was the same as that on the controls (0.1 per cent.). But when 13 trees that had been

TABLE I

PERCENTAGES OF CITRUS FRUITS HAVING RIDGES AND SECTORS IN THE RIND, FROM TREES FUMIGATED WITH HCN AT DIFFERENT TIMES OF YEAR AND FROM UNFUMIGATED TREES

GROVE	VARIETY OF CITRUS	NUMBER OF TREES	HCN FUMIGATION		DATE OF SAMPLING	MEAN PERCENTAGE OF FRUITS RIDGED†
			HCN PER UNIT*	DATE		
			ml.			%
1	Navel orange	26 (Control)			Dec. 11, 1940	0.1
		24	24	Feb. 24, 1940	Dec. 11, 1940	36.0
		18	20	Nov. 27, 1939	Dec. 11, 1940	0.1
		13‡	{20 / 24}	Nov. 27, 1939 / Feb. 24, 1940	Dec. 11, 1940	36.6
2	Navel orange	6 (Control)			Jan. 3, 1941	0.1
		8	20	Dec. 14, 1939	Jan. 3, 1941	0.8
		8	22	Feb. 21, 1940	Jan. 3, 1941	24.3
	Valencia orange	8 (Control)			Jan. 3, 1941	0.1
		7	20	Dec. 14, 1939	Jan. 3, 1941	0.2
		8	22	Feb. 21, 1940	Jan. 3, 1941	31.4
3	Navel orange	11 (Control)			Aug. 14, 1941	0.0
		11	24	Feb. 4, 1941	Aug. 14, 1941	28.3
4	Grapefruit	21 (Control)			Jan. 3, 1941	0.2
		25	24	Feb. 16, 1940	Jan. 3, 1941	22.1
5	Lemon	8 (Control)			Mar. 11, 1941	0.2
		14	28	Feb. 26, 1940	Mar. 11, 1941	40.1

* A unit in citrus fumigation is approximately 100 cu. ft. for the average-sized tree.
† Percentages based on counts, at random, of 200 fruits per tree.
‡ These trees were fumigated twice, once on Nov. 27, 1939, and again on Feb. 24, 1940.

fumigated on November 27, 1939, were fumigated a second time, on February 24, 1940, the percentage of ridged fruits produced was 36.6. These experimental data therefore demonstrate that navel orange trees can be fumigated in November without increasing the amount of ridged fruit.

Grove 2 consisted of both navel and Valencia orange varieties, planted alternately in the rows. Six trees served as controls for the navel plot, and 8 trees served as controls for the Valencia plot. Each of the control plots contained 0.1 per cent. ridged fruits. In the navel plot, two sets of 8 trees each were fumigated, one on December 14, 1939, and the other, February 21, 1940. In the Valencia plot, 7 trees were fumigated December 14, 1939, and 8 trees were fumigated February 21, 1940. In the two groups of trees that were fumigated December 14, 1939, the navels had 0.8 per cent. and the Valencias 0.2 per cent. ridged fruits. In the two groups of trees that were fumigated February 21, 1940, the navels had 24.3 per cent. and the Valencias 31.4 per cent. ridged fruits. In this particular grove, it can be seen that the ridging of the fruit on the trees fumigated in February was vastly significant, whereas the ridging of the fruit on the trees fumigated in December was of the same order of magnitude as that of the controls.

The trees in grove 3 were fumigated February 4, 1941, and the fruit was counted for the amount of ridging on August 14, 1941. As these trees were of the navel variety, the size of the fruit at the date of counting ranged from ½ to 1 inch in diameter. The fumigated trees showed an average of 28.3 per cent. of ridged fruit, while the control trees showed none at all.

Some experimental data were obtained from grapefruit trees (grove 4) fumigated February 16, 1940. The mean percentage of ridged fruit on 25 fumigated trees was 22.1, and the mean percentage of ridged fruit on the 21 control trees was 0.2.

Lemon trees (grove 5) that were fumigated February 26, 1940, fruits of which were counted on March 11, 1941, showed 40.1 per cent. ridged fruits. The untreated trees in this grove had 0.2 per cent. of their fruits affected.

Discussion

The data of table 1 show that fumigation of citrus trees with HCN, when the fruit was in a certain stage of bud development, resulted in the formation of sectors and ridges in the outer peel of the fruits of navel and Valencia orange, grapefruit, and lemon varieties. The time of year in which ridging will occur varies somewhat with the different varieties of citrus. In the navel and Valencia orange and in the grapefruit varieties, the highest percentage of fruits affected was from the trees fumigated in the month of February, although in some groves extreme ridging of the fruits has been observed to occur on trees fumigated the latter part of January. Extensive field observations have shown that fruits from lemon trees become severely affected if the trees are fumigated any time from late January to April, inclusive. Fruit buds of lemon trees develop at different

times of year, but the largest setting of fruit occurs in the spring over a
prolonged period.

The ridging of citrus fruits may therefore be said to depend upon the
stage of development of the fruit buds at the time the trees are fumigated.
Variation in the time and rate of bud development in different groves and
in different years indicates a certain variation in the time of year in which
fumigation will cause the development of ridging in the fruit.

The form of the rind thickenings is very suggestive of that found in the
well-known sectorial chimeras which are frequent in citrus (2). It is pos-
sible that the change caused by HCN often affects one or more cells in the
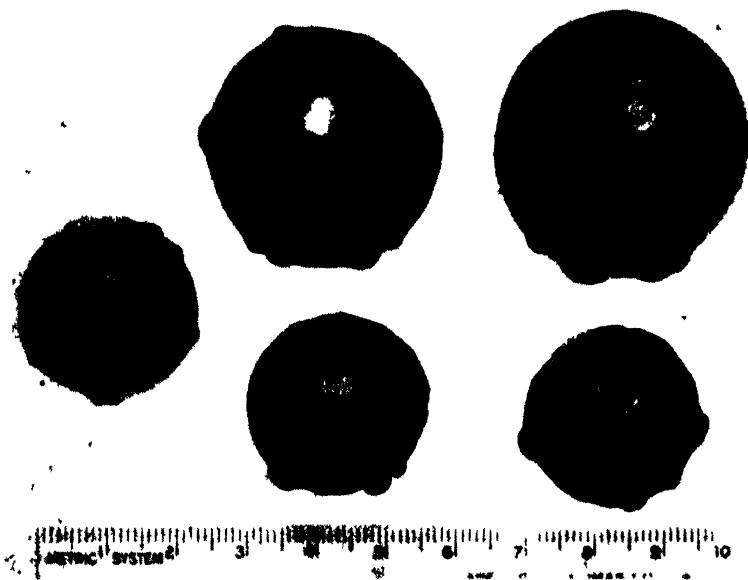developing ovary before cell division is ended. This change may be trans-

FIG. 3. Ridges on small, immature navel oranges selected from trees which had 28.3
per cent. of their fruits affected (Grove 3, table I).

mitted in the process of mitotic cell division and result in the formation of
sectors originating from one cell or from a relatively small number of
cells. Whether rind thickening is due to an increase in cell number or in
cell size has not been determined. It may be that HCN causes a genetic
change, such as a doubling of the number of chromosomes. Sectors of
modified tissue appear in plants after treatment with colchicine, owing to
the presence of tetraploid sectors, as in Lilium (1).

Just what effect climatic conditions have on the severity of the ridging
of citrus fruits when fumigated with HCN is not known. It has been sug-
gested by some workers that a warm, mild winter contributes to the ridging
of fruits, but no experimental data are available on this point. Climatic
and seasonal changes undoubtedly affect the time and rate of bud develop-

ment; but an important factor in producing this phenomenon in fruits appears to be the definite stage of bud development at the time of fumigation. *If the trees are fumigated prior to or subsequent to this particular and definite stage in development of the bud, only the natural percentage (0.1 to 1.5) of ridged fruits will result.* According to our experimental results and field observations, HCN affects only the fruit that is in the bud stage of development at the time of fumigation; the fumigation of the trees after the fruit has formed does not increase the amount of ridged fruit.

The effect of the HCN does not carry over to the following year. As an illustration, trees that were fumigated on February 26, 1940, had an average of 25 per cent. of ridged fruit for that crop. These trees were not fumigated in February, 1941, and the green fruit from the buds of the suc-
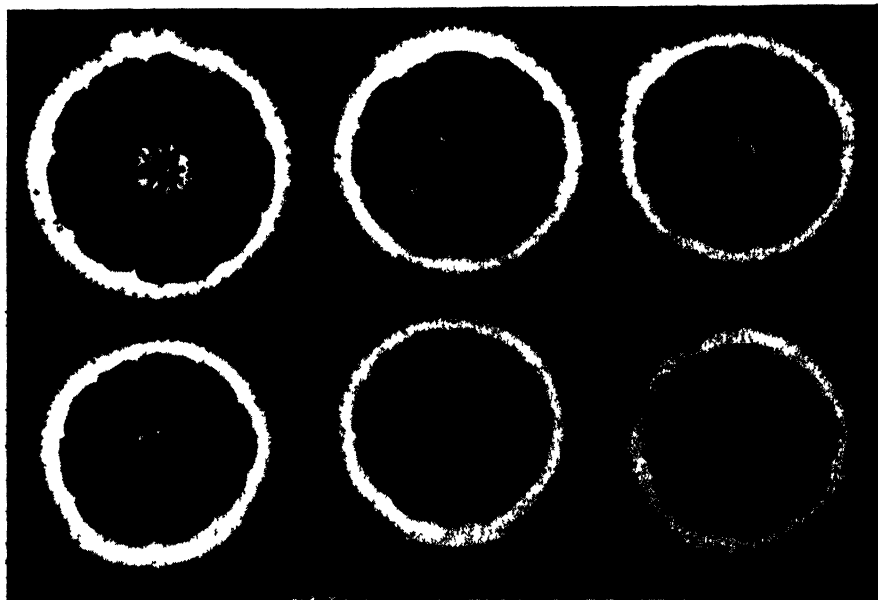

FIG. 4.   Cross sections of mature Valencia oranges, showing that the location of ridges on the fruit surface is in no way correlated with the arrangement of fruit segments.

ceeding crop showed no ridging. Observations of this kind were made on many groves in which both crops of fruit, mature and immature (green), were on the trees at the same time.

The appearance of ridges and sectors after fumigation is not correlated with size of the fruit. Ridging is about equally distributed, occurring on fruit of all sizes on the tree. In fact, the ridges appear on the very small, immature fruit (fig. 3) and they persist throughout its growth and maturation. This phenomenon appears to be confined solely to the outer peel, the flavedo (fig. 4). The physical appearance of the albedo, and of segments of affected fruit, is normal in every respect. The location of the ridges on the fruit surface is not in any way correlated with the arrangement of the segments inside the fruit (fig. 4).

Trees in a given grove, fumigated at the same time, varied as much as 100 per cent. in the number of fruits having ridges and sectors. When the amount of affected fruit on the fumigated trees was compared with that on the controls, the least amount of ridged fruit on the fumigated trees was, in every instance, more than ten times the greatest amount on the control trees. In accord with such experimental results, it is reasonable to conclude that the fumigated trees had a significantly greater amount of ridged fruits than the controls.

Conclusions

The fumigation of citrus trees, when the fruit buds are in a certain stage of development, induces ridges and sectors in the outer peel (flavedo) of the fruit.

In the navel orange, Valencia orange, and grapefruit varieties, fumigation of trees in February produced the greatest amount of ridged fruits, but in some years, extreme ridging of the fruits occurred on trees fumigated the latter part of January.

In the lemon variety, trees fumigated in February produced the highest percentages of fruits with ridges and sectors, but extensive field observations have shown that fruits of lemon trees fumigated from late January to April, inclusive, were severely affected.

Variation in the time and rate of bud development of citrus trees in different groves and in different years indicates a definite variation in the time of the year in which ridging will be produced in the fruit by fumigation. The development of ridges and sectors on citrus fruits depends mainly upon the stage of development of the buds at the time the trees are fumigated.

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LITERATURE CITED

1. EMSWELLER, S. L., and BRIERLEY, PHILIP. Colchicine-induced tetra-ploidy in Lilium. Jour. Hered. **31**: 223–230. 1940.

2. FROST, HOWARD B. Polyembryony, heterozygosis and chimeras in citrus. Hilgardia **1**: 365–402. 1926.

3. LINDGREN, DAVID L., and SINCLAIR, W. B. The ridging of citrus fruits as influenced by fumigation of citrus trees with HCN. Jour. Econ. Ent. **34**: 477. 1941.

4. SHAMEL, A. D., POMEROY, CARL S., and CARYL, R. E. Bud selection in the Washington Navel orange: progeny tests of limb variations. U. S. Dept. Agr. Tech. Bull. 123. 1929.

OXIDATION–REDUCTION POTENTIALS OF THE TISSUE FLUID OF CABBAGE[1]

ELLIS AIROLA AND JOHN W. CRIST

(WITH TWO FIGURES)

Introduction

It has been stated (2) "that oxidation-reduction systems play so intimate and so essential a part in living organisms that life itself may be defined as a continuous oxidation-reduction reaction."

CANNAN (1), in 1926, investigated the electrode potentials of extracts obtained from the green shoots of *Mercurialis perennis*. Work of this kind, with higher plants—making use of the electrometric method—has not been extensive since the appearance of his publication. Additional effort in this field of investigation seemed to be warranted.

Materials and methods

The plant selected for use was cabbage (*Brassica oleracea*), in three of its leading commercial varieties: Ferry's Round Dutch, Large Wakefield, Early Jersey Wakefield.

The seeds for each successive lot of plants were germinated in moist sand. The seedlings, after emergence of the first leaves, were transplanted to four-inch pots. The soil of the pots was a homogeneous mixture of two-thirds compost and one-third sand. The pots, while the plants grew to the size required for sampling, were kept buried in moist sand on a greenhouse bench, and the percentage of moisture in them held essentially constant and at the optimum for the plants' need.

SAMPLING

When they had reached the desired size, the plants were cut off just above the soil surface; placed with their cut ends in water, and taken to the laboratory, where the leaves were quickly removed. Selection was made only of whole, well or fully developed, leaves, exclusive of their petioles, for the extraction of the tissue fluid. The size of the sample approximated 20 grams.

EXTRACTION

The selected leaves were put at once into a beaker and covered with diethyl ether. The time for their plasmolysis was a period that ended just before the cell sap began to exude. This period was found to vary with the ages of the leaves, and, from preliminary tests, was observed to be around thirty seconds for the younger leaves and one minute for the older ones. The plasmolyzed leaves were removed from the beaker; allowed to dry off on paper toweling; were wrapped in a prepared canvas cloth; placed in a

[1] Journal Article no. 593 (n.s.), Michigan Experiment Station.

nickel-plated brass cylinder; and extracted by means of an hydraulic press at a uniform pressure of 800 lb. per sq. in., with a uniform period of drainage of 45 seconds. The cylinder was of such design that the expressed juice could be drawn off into a pipette as rapidly as it appeared. This reduced the possibility of its oxidation from exposure to the air.

INSTRUMENTAL MEASUREMENTS

The pH of the extracted juice was determined immediately by means of a Cameron (glass electrode) hydrogen-ion meter. The sample was then transferred to the oxidation-reduction cell, containing a glass-tube inlet through which bubbling oxygen-free nitrogen was admitted. Two platinum electrodes, in addition to the half-cell, dipped into the extract.

Preliminary tests showed that the platinum electrodes required from 4 to 6 minutes to come to equilibrium with the surrounding solution. This time was used to adjust the current of the amplifier (Leeds & Northrup, no. 7673, Thermionic) and also that of the standard cell, in the potentiometric system, to zero. With these zero points attained, the E.M.F. leads from the half-cell and one of the platinum electrodes were connected through the amplifier and the first reading taken. A duplicated reading was then made with the second, instead of the first, electrode connected in the circuit. These two readings, which in nearly all instances were less than three millivolts apart, were averaged. This average, after reference to a normal hydrogen electrode, was taken to be the oxidation-reduction potential of the extract.

TREATMENT OF ELECTRODES

Several methods for cleaning and restoring the electrodes were tried. Of these the most satisfactory way proved to be their submersion in fusing potassium acid sulphate, from which they were afterwards dissolved free in distilled water, and then let stand in distilled water until the next set of measurements was to be made.

Results

RELATIONSHIP BETWEEN pH AND Eh VALUES

Three separate sets of determinations, aimed to discover this relationship, were completed. The plants were of the Ferry Round Dutch variety and were respectively 43, 45, and 46 days old for the three tests.

The manner of testing the extracted sample was first to determine its pH value, and then its Eh value. Following this, it was returned to the pH receptacle and the desired amount of 0.1 N HCl added to it, whereupon its pH and Eh values were redetermined. This process was continued, with successive introductions of added amounts of the acid. The data appear in table I.

From inspection of table I the existence of a systematic relationship between pH and Eh seems to be evident. This relationship in the tests

TABLE I

EFFECT OF pH ON EH (IN MILLIVOLTS) OF CELL SAP

TEST 1		TEST 2		TEST 3	
pH	Eh	pH	Eh	pH	Eh
5.80*	+ 453*	5.78*	+ 471*	5.82*	+ 456*
4.30	+ 504	4.30	+ 520	4.85	+ 496
3.70	+ 527	3.80	+ 534	4.53	+ 504
3.30	+ 549	3.40	+ 548	3.50	+ 538
2.30	+ 587	3.10	+ 570	3.08	+ 555
1.70	+ 629	2.50	+ 581	2.80	+ 562
1.45	+ 660	1.97	+ 626	2.35	+ 610
1.20	+ 678	1.60	+ 662	2.05	+ 615
		1.39	+ 681		
		1.29	+ 691		

* Original extract.

proved to be curvilinear in character. The curve of best fit in each test was parabolic in type. When the measurements of all three of the tests were combined, and plotted, the result shown in figure 1 was obtained. The equation of the fitted curve is: $Y = 799.96 - 103.761\,X + 8.0937\,X^2$.

LEAF POSITION AND EH VALUES

It became a matter of interest to determine whether or not the oxidation-reduction potentials of the tissue fluid varied significantly within the plant
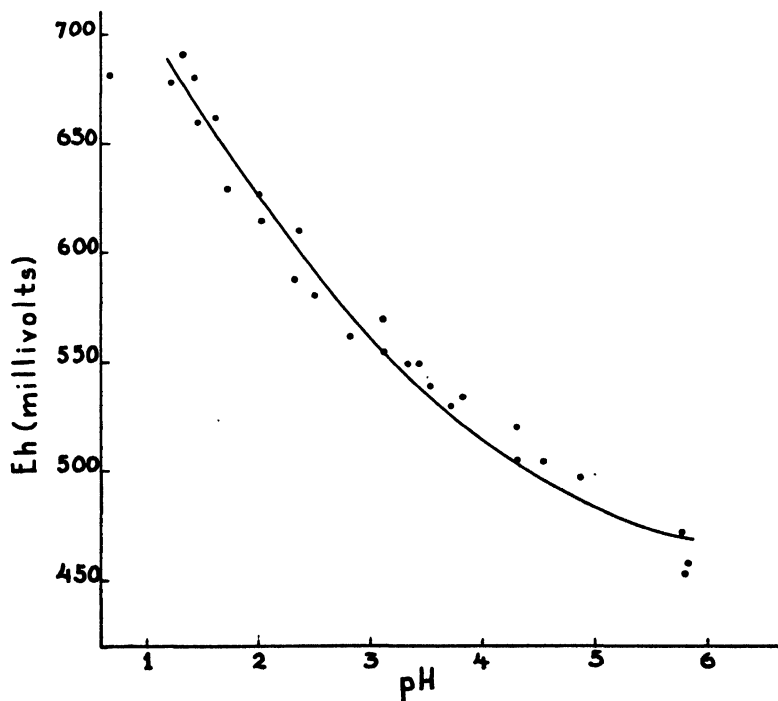

FIG. 1. Relationship between pH and Eh values of tissue fluid.

itself. Tests for this were made with respect to the positions of the leaves on the stem, for the Early Jersey Wakefield variety of cabbage.

Three such tests were completed. The plants were respectively 42, 44, and 46 days old, and were in the stage of each having 8 or 9 leaves. In each case, 9 plants, of uniform size and vigor, were selected and used. From these 9 plants the second leaves (ones next below emerging leaves) were taken and combined for use as a sample. The same procedure was followed for the third, fourth, fifth, and sixth orders of leaves, down the stem. The data appear in table II.

These tests (data in table II), based upon leaf-position, were essentially a consideration of the ages of the leaves. The sampling in each case ranged, through a series of grouped specimens, from juvenile, to virtually mature, to more or less senile leaves.

The data give some indication of systematic variance. The Eh values appear to increase with the development of the young leaf; reach maxima

TABLE II

EH VALUES (IN MILLIVOLTS) FOR CELL SAP FROM DIFFERENT ORDERS OF LEAVES

ORDER OF LEAVES	TEST 1	TEST 2	TEST 3	MEAN
	Eh	Eh	Eh	Eh
2nd	+ 439	+ 453	+ 455	+ 449
3rd	+ 464	+ 469	+ 462	+ 465
4th	+ 453	+ 460	+ 469	+ 461
5th	+ 450	+ 460	+ 461	+ 457
6th	+ 447	+ 458	+ 450	+ 452

(3rd or 4th leaf), and beyond this tend to decrease with increasing age of the leaf. The differences, while mostly consistent in order, are, however, small in magnitude, and mostly insignificant in a statistical sense. Assuming them to be real, many more observations would be required certainly to establish significance.

EFFECT OF HARDENING ON EH VALUES

It was of interest to ascertain whether or not the oxidation-reduction system of the cabbage plant would prove to be sensitive in its equilibrium to a change in the plant's environment. Exposure to low temperature (hardening) was chosen for trial.

One experiment was conducted with plants of the Ferry's Round Dutch variety; three with Ferry's Large Wakefield variety. In each instance, the plants were grown, as heretofore described, to a size adequate for use. At this point, which varied from 44 days for Ferry's Round Dutch to 35, 43, and 37 days respectively in the three tests with Ferry's Large Wakefield, the lot of plants was divided into two groups of selected and equal size and vigor. One lot was to be subjected to hardening, the other not (left continuously in the greenhouse).

PROCEDURE FOR HARDENING.—The lot of plants to be hardened was removed each day at 6 P.M. from the greenhouse to a cold storage room with a regulated temperature of 7° to 8.5° C. It was left there until 6 A.M. (a period of 12 hours), and then shifted back into the greenhouse. This was repeated for 6 consecutive nights.

Sample plants, for laboratory use, were taken each morning from each of the two lots between 9 and 10 A.M. The period from 6 A.M. to 9 A.M. sufficed for a return of the plants in the treated lot to the higher temperature of the greenhouse (16° to 22° C.).

The type of the results for the four experiments was essentially the same. It suffices, therefore, to present only the result from one experiment—that
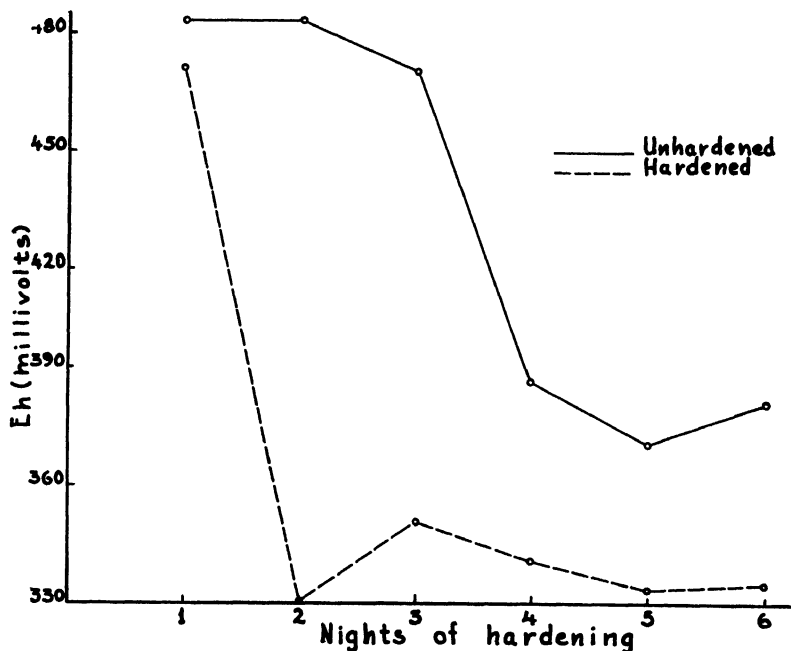

FIG. 2.   Effect of hardening on Eh values of tissue fluid.

for Ferry's Large Wakefield variety—conducted in June, 1941. It is given by figure 2.

A decline in the Eh values of both sets of plants is shown by figure 2. For the untreated plants it amounts, by steps, to about 100 millivolts. For the treated plants the decline is much greater and, furthermore, appears to be somewhat different in type.

The low point (+ 330 m.v.), reached after the second night of the plants' exposure to low temperature, is of special interest. A point of this nature appeared in each one of the four tests. The only variation was the time of its appearance. In the other two tests with this variety of cabbage, which were made in April, 1941, it appeared after the fourth night of exposure rather than the second, as shown here.

In all cases this low point marked the maximal difference between the Eh values for the two sets of plants. After it was reached and passed, a substantial difference in these values continued but was lower in magnitude. Prolongation of the experiment, with additional nights of treatment, was desired but could not be effected.

Discussion

Among the findings herein reported, the most important may possibly be that of the sharp decline of the oxidation-reduction potential in response to the hardening treatment. This shift of the potential was to a remarkably lower value, meaning a sudden-and large relative increase of reduction over oxidation. Doubtless this involved a change in energy relations, since the oxidation-reduction systems of biological organisms appear to be of the nature of thermodynamically reversible reactions which are characterized by electronic migrations.

Incidentally, it was noticed without exception that coincidental with the sharp drop in the potential of the treated plants there was a change in the color of the expressed tissue fluid. It was emphatically bluish-purple instead of green. This color persisted and became slightly more intense with the additional exposures of the plants to the low temperature. The leaves, in external appearance, had this same caste of color. Its general appearance in cabbage plants, undergoing hardening to cold, is an item in common experience. This is suggestive of the presence of a chromogen which may be involved in the plant's oxidation-reduction system, and which, as such, would bear particular investigation.

Summary

1. The existence of an oxidation-reduction system in the tissue fluid of the cabbage plant was experimentally demonstrated. Its electric potential, with normally grown plants, was found to be positive in character (dominance of oxidation over reduction) and to vary within the range of $+450$ to $+475$ millivolts.

2. The relationship between the pH and the Eh of the tissue fluid was found to be definite and regular. It was expressible in the mathematical form of a parabola, the equation of which was derived, and is presented.

3. Tests were made for the effect of leaf position (essentially leaf age) on the plant's oxidation-reduction potential. Although inadequate as proof, the evidence pointed to the potential being low in the young leaves; highest in the leaves at full development; and declining in magnitude as the leaves passed the stage of maturity and began to age.

4. The oxidation-reduction system of the cabbage plant gave an apparent response to the condition of exposure of the plant to low temperature (hardening). This response, consistent within the limits of the experimentation, was of the nature of a sharp drop in the potential after two to four exposures to low temperature; a slight rise in the Eh values, with added exposures;

and their continuation at a level below that of the unexposed and unhard-
ened plants.

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LITERATURE CITED

1. CANNAN, R. K. Electrode potentials of hermidin, the chromogen of
        Mercurialis perennis. Biochem. Jour. **20**: 927–937. 1926.
2. HEWITT, L. F. Oxidation-reduction potentials in bacteriology and bio-
        chemistry. London County Council (England), 4th ed., 1936.

THE USE OF ION EXCHANGE MATERIALS IN STUDIES ON CORN NUTRITION[1]

C. D. CONVERSE, NATHAN GAMMON, AND
J. D. SAYRE

(WITH THREE FIGURES)

Introduction

In 1937, a series of studies involving the use of ion-adsorbing, or ion-exchange materials was begun at the Ohio Agricultural Experiment Station in an effort to overcome some of the difficulties previously encountered in growing corn to maturity in controlled nutrition experiments. Nutrient culture techniques involving the use of these materials present many advantages over those in present use, particularly over solution cultures. Plants can be grown to full size and maturity under carefully controlled conditions. The technique permits the study of typical mineral deficiency symptoms and responses to varying amounts of the different elements. More accurate studies of the effect of the relative mineral content of the nutrient media on the mineral content of the plant parts are possible. Single elements can be varied without changing over ion concentrations. Undesirable osmotic pressures are eliminated. Changes in pH of the cultures are reduced to a minimum, and mutual precipitation of ions from solutions is avoided. The time required to care for the cultures is greatly reduced, as fresh solutions are not required at frequent intervals. The time required to set up the original cultures is somewhat greater than for solution cultures, because it is necessary to prepare different ion-exchange materials in rather large quantities in advance. Since the exchange materials must be intimately mixed with the gravel, it is not possible to change the nutrient levels of the cultures very much after they are started; thus, any mistakes in supply of the different elements cannot be corrected, nor can any addition or subtraction be made after the cultures are started. This might be a disadvantage if different conditions of nutrient supply were required for the different stages of growth of the plant.

The problem of growing plants in solution cultures has received considerable attention, and there are very extensive bibliographies of the literature on this subject. Few investigators, however, have used adsorbed ions for growing plants. Pioneer work on this subject has been done by BREAZEALE (4) and McCALLA (7), who have studied artificial zeolites and the clay fraction from the soil and used them as a source of ions for plants. ALBRECHT and McCALLA (1) give a very good account of this subject and references to previous work.

SCHLENKER (8) began work at the Ohio Agricultural Experiment Station

in 1938 and carried it on at the Connecticut Agricultural Experiment Station with the same type of materials.   A progress report covering the Ohio work to date was read at the meeting of the American Society of Plant Physiologists at Columbus, Ohio, in December 1939 (5).

Materials and methods

Materials were prepared during the winter of 1937–1938, and in the summer of 1938 corn was grown to full maturity in a large gravel tank using an iron-phosphorus-bentonite sorption complex as the sole source of iron and phosphorus.   At the same time, a series of pots was set up, using zeolite with all ions adsorbed on it, supplemented with additional iron bentonite, and irrigated with distilled water.   The corn in these pots grew well and, of necessity, was harvested when ear shoots were formed, as it was planted rather late.   Other work of a preliminary nature, using the colloidal fraction of a Miami subsoil as a sorption complex, was begun at this time but was abandoned in favor of commercial water-softening materials, which, because of their granular nature, were much easier to prepare and handle than the bentonite and clay colloids.   Although iron has been supplied successfully on the various adsorption agents, it has been used more conveniently in the form of finely divided magnetite, as suggested by EATON (6).

The ion-adsorbing materials used in these studies included bentonite,[2] exchange capacity, 80 mg. equivalents per 100 gm.; Miami subsoil clay colloid, 65 m.e., prepared by BRADFIELD's method (2, 3) and electrodialyzed free of positive ions other than hydrogen; "Decalso,"[3] an artificial sodium zeolite, 230 m.e.; "Zeo-Karb-H,"[3] a granular base-free hydrogen exchange material made from coal, 200 m.e.; and "De-Acidite,"[3] an aniline-anion-fixing material, 120 m.e.

The iron-phosphorus-bentonite complex was prepared by dispersing the bentonite in distilled water and adding an excess of a ferric sulphate solution.   After thorough mixing, the supernatant liquid was siphoned off, and more distilled water and ferric sulphate were added.   This process was repeated, and the mixture of bentonite and ferric sulphate was washed with distilled water until the bentonite no longer settled out.   This procedure produced an iron-bentonite.   An iron-phosphorus-bentonite complex was formed by adding phosphoric acid to this mixture, and the process of saturating and washing was repeated.   Finally, the excess liquid was removed by supercentrifuging, and the resulting paste was stored in glass jars.

The hydrogen clay was dispersed to about a 2 per cent. suspension and treated with the hydroxide or the carbonate of the desired element in accordance with its equivalence value as determined by electrometric titration.

The commercial materials are granular and were treated without any previous preparation.   The Decalso was leached with a solution of the chloride salt of the desired cation until saturated.   The Zeo-Karb-H was

[2] Furnished by the Wyodak Chemical Co., Cleveland, Ohio.
[3] Furnished by the Permutit Co., New York.

treated in a water suspension with the hydroxide or carbonate of the element desired, in accordance with its equivalence value as determined electrometrically. The De-Acidite was shaken with an excess of dilute acid of the anion desired and permitted to stand overnight. Following treatment, excess salts were washed out with distilled water on a suction filter, after which the materials were dried.

All materials were analyzed after treatment to determine the amounts of ions adsorbed. Two-gm. samples were leached with 200 ml. of normal neutral ammonium acetate, and the leachate was analyzed for the adsorbed ion by standard methods to check the quantity of ions adsorbed.

The amounts of the elements necessary in the culture media to mature a single corn plant were determined from previous analyses of both field- and solution-grown corn plants. When the quantity of the elements adsorbed on the exchange materials was known, it was simple to weigh out enough of the materials to supply the necessary elements.

At planting time, the pots and reservoirs were assembled as shown in the diagram (fig. 1). Twelve hundred gm. of no. 4 clean quartz gravel were
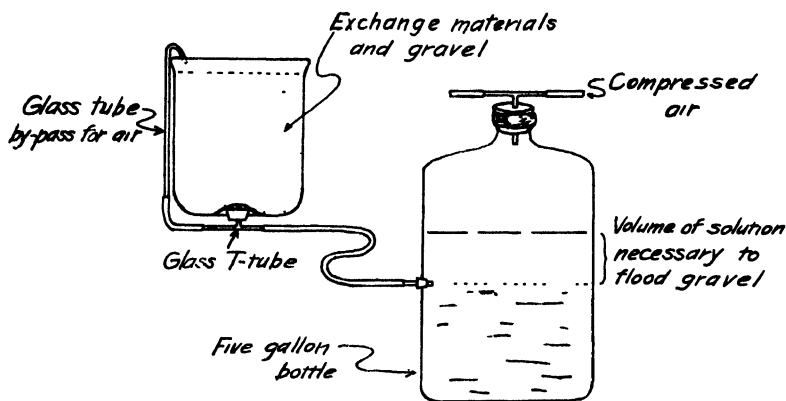

FIG. 1. Sketch of the pots and reservoirs used in cultures of ion exchange materials.

weighed out for each pot, put into a 10-gallon barrel churn with 200 ml. of rain water and the desired quantities of exchange materials, and mixed for 5 minutes. It was found that a 3-inch baffle board fitted diagonally from top to bottom in the churn gave a thorough mix.

The pots were irrigated automatically four times daily. Seed was planted about an inch deep, and when the plants were about 4 inches high, they were thinned to one stalk at a place. Tillers were removed if they developed. From one to four stalks of corn were grown in a pot, although it was found that when four stalks were grown, the total dry weight produced per plant was reduced, apparently because of root-binding. Water which evaporated or transpired from the pots was replaced daily with rain water.

Results

This type of culture has been used satisfactorily to study growth re-

sponses and deficiency symptoms of corn to calcium, magnesium, potassium, phosphorus, nitrogen, sulphur, and boron.   Typical results obtained by this method are illustrated by two experiments in which series of levels of potassium and phosphorus were used.   Two plants were grown in each culture,
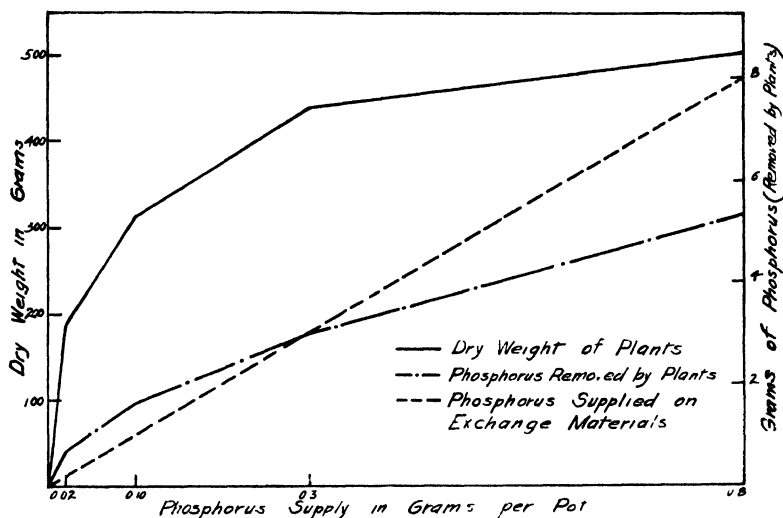

Fig. 2.   Dry weight production by the two plants in each culture and phosphorus accumulation in the four levels of supply.
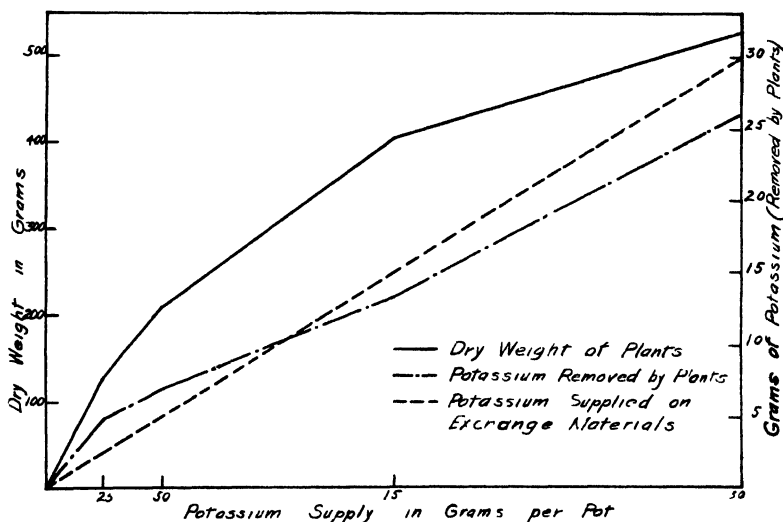

Fig. 3.   Dry weight production by the two plants in each culture and potassium accumulation in the four levels of supply.

one each of the two single crosses WF9 × 38–11 and 56 × 4–8.   The series were based on four levels of supply of either potassium or phosphorus; all other nutrients were kept constant.   The potassium series was replicated three times, and the phosphorus series twice.   The general growth and appearance of the plants in the higher levels of nutrition were similar to those

TABLE I

RESULTS OF CHEMICAL ANALYSIS OF PLANTS GROWN AT THE FOUR LEVELS OF SUPPLY OF POTASSIUM, SUMMER 1940. EXPRESSED ON A DRY WEIGHT BASIS

ELEMENT ADDED IN THE EXCHANGE FORM	LEVEL 1			LEVEL 2			LEVEL 3			LEVEL 4		
	AMOUNT ADDED PER POT	AMOUNT REMOVED PER POT	CONCENTRATION IN PLANT	AMOUNT ADDED PER POT	AMOUNT REMOVED PER POT	CONCENTRATION IN PLANT	AMOUNT ADDED PER POT	AMOUNT REMOVED PER POT	CONCENTRATION IN PLANT	AMOUNT ADDED PER POT	AMOUNT REMOVED PER POT	CONCENTRATION IN PLANT
	$gm.$	$gm.$	%	$gm.$	$gm.$	%	$gm.$	$gm.$	%	$gm.$	$gm.$	%
Potassium	0.25	0.48	0.38	0.50	0.69	0.33	1.50	1.31	0.32	3.00	2.63	0.50
Phosphorus	1.00	0.236	0.184	1.00	0.205	0.097	1.00	0.342	0.084	1.00	0.413	0.078
Nitrogen*	6.00	3.01	2.35	6.00	3.31	1.57	6.00	4.16	1.02	6.00	4.62	0.87
Calcium	2.50	0.71	0.55	2.50	0.81	0.38	2.50	1.19	0.29	2.50	1.02	0.19
Average dry matter produced per pot of two plants, gm.	128			211			406			500		

* Part of the nitrogen was supplied in solution as urea. The minor elements, except iron, were supplied in dilute solutions.

TABLE II

RESULTS OF CHEMICAL ANALYSIS OF PLANTS GROWN AT THE FOUR LEVELS OF SUPPLY OF PHOSPHOROUS, SUMMER 1940. EXPRESSED ON A DRY WEIGHT BASIS

ELEMENT ADDED IN THE EXCHANGE FORM	LEVEL 1			LEVEL 2			LEVEL 3			LEVEL 4		
	AMOUNT ADDED PER POT	AMOUNT REMOVED PER POT	CONCENTRATION IN PLANT	AMOUNT ADDED PER POT	AMOUNT REMOVED PER POT	CONCENTRATION IN PLANT	AMOUNT ADDED PER POT	AMOUNT REMOVED PER POT	CONCENTRATION IN PLANT	AMOUNT ADDED PER POT	AMOUNT REMOVED PER POT	CONCENTRATION IN PLANT
	gm.	gm.	%	gm.	gm.	%	gm.	gm.	%	gm.	gm.	%
Phosphorus	0.02	0.074	0.039	0.10	0.162	0.052	0.30	0.296	0.067	0.80	0.525	0.104
Potassium	5.00	2.13	1.13	5.00	2.79	0.89	5.00	2.99	0.68	5.00	2.75	0.55
Nitrogen*	6.00	2.63	1.40	6.00	3.59	1.15	6.00	4.08	0.93	6.00	4.23	0.84
Calcium	2.50	0.21	0.11	2.50	0.28	0.09	2.50	0.44	0.10	2.50	0.55	0.11
Average dry matter produced per pot of two plants, gm.	188			313			439			503		

* Part of the nitrogen was supplied in solution as urea. The minor elements, except iron, were supplied in dilute solutions.

of field-grown plants, whereas typical deficiency symptoms developed in the lower levels.

Distinct differences were shown between the two hybrids used in these experiments, but the results given in this paper are average values. They are shown graphically in figures 2 and 3. The total dry weight production per culture of the above-ground portion of the plants increased progressively with increasing nutrient supply, and the amount of phosphorus and potassium accumulated by the plants increased also, but not in proportion to the amount supplied in the cultures. In fact, the results show that the plants removed more than was supplied at the lower levels, probably because of contamination of the cultures from dust or from the rain water used.

Tables I and II give a brief summary of the results of the chemical analysis of the plants grown in these two experiments and a record of the amount of each of the major elements supplied to each culture.

Discussion

None of the major elements were limiting factors except where made so, because the total amount of the element absorbed was only a small part of the amount added. A relationship between calcium and potassium is shown in table I, which records how the increased potassium absorbed by the plants reduced the percentage concentration of calcium in the plants. This is not a reciprocal relationship because the total amount of calcium was not depressed except in the last level. Relationships exist among the other elements, but it is difficult to interpret them because of the great change in plant size which occurred with increased supplies of potassium.

The results shown in table II, showing an experiment in which all elements except phosphorus were constant, are also difficult to interpret. Phosphorus increased in amount and in concentration in each level of supply. Potassium decreased in concentration as the plants became larger, but the total amount taken up increased except in the last level. Nitrogen was absorbed by the plants in greater amounts in each level but not in proportion to plant size, because the concentration decreased in each level. Calcium changed significantly in the different levels; it was very low in all.

Summary

A new technique for plant nutrition studies has been outlined, and illustrations of the type of results that may be obtained are given. This new technique permits the addition of each major element separately. Growth responses and deficiency symptoms are obtained just as easily as in solution cultures, but the responses and symptoms are more definite than in other methods of culture.

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LITERATURE CITED

1. ALBRECHT, WM. A., and MCCALLA, T. M. The colloidal clay fraction of soil as a cultural medium. Amer. Jour. Bot. 25: 403–407. 1938.

2. BRADFIELD, R.   The chemical nature of colloidal clay.   Missouri Agr. Exp. Sta. Res. Bull. 60.   1923.

3. ——————.   An inexpensive cell for purification of colloids by electro-dialysis.   Ind. & Eng. Chem. **20**: 79.   1928.

4. BREAZEALE, J. F.   Soil zeolites and plant growth.   Arizona Agr. Exp. Sta. Tech. Bull. 21.   1928.

5. CONVERSE, C. D., and GAMMON, JR., NATHAN.   The use of ion exchange materials in physiologic studies of corn.   Preliminary report read before the American Society of Plant Physiologists.   December 30, 1939.

6. EATON, F. M.   Automatically operated sand-culture equipment.   Jour. Agr. Res. **53**: 433–444.   1936.

7. McCALLA, T. M.   Behavior of legume bacteria in relation to exchangeable calcium and hydrogen-ion concentration of the colloidal fraction of the soil.   Missouri Agr. Exp. Sta. Res. Bull. 256.   1937.

8. SCHLENKER, FRANK S.   Plant growth in culture solutions and availability of ions adsorbed on Permutit and Aniline Black.   Amer. Jour. Bot. **27**: 525–529.   1940.

ESTIMATION OF STOMATED FOLIAR SURFACE
OF PINES

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(WITH ONE FIGURE)

Physiological investigations of forest tree species require, on occasion, the measurement of the total surface area of tree foliage. The problem of determining leaf surface areas of broad-leaved species is not as difficult as that of determining foliage surface area in coniferous species; for despite individual irregularity, leaves of broad-leaved species may be traced and their outlines planimetered. An improved technique for determining exposed leaf area of broad-leaved species is the photoelectric cell method, one modification of which is described by KRAMER (5). The grid-area method of NEWMAN (7) which is an application of the mean ordinate principle can also be used for leaves of broad-leaved species. These methods cannot be applied to the needle-like leaves of pine.

GROOM (2) estimated the surface of coniferous foliage by combining his measurements of numbers and lengths of leaves with those of VON HÖHNEL (3, 4). MINCKLER (6) determined leaf area in pine by taking diameter measurements with an eyepiece micrometer and making a small allowance for taper. UHL (8) adopted the displacement principle and suggested a relationship between surface development and volume but did not give information on taper corrections.

This paper describes an indirect method of measuring the aggregate surface area of pine needles by taking advantage of the correlation between surface area and volume of needle fascicles; for the latter may be determined directly and quickly by the measurement of displacement.

The method involves the following assumptions with respect to the cross-section of a pine needle fascicle when the component needles are in properly-oriented contact:

1. That the outline of any cross-section along the length of fascicle is circular; that departure therefrom gives rise to no greater relative error of calculated surface or volume of the fascicle than does the corresponding assumption on the calculated surface or volume of the tree bole; in short, that the consequence of departure from circular outlines is negligible.

2. That contiguous faces of needles, as viewed in cross-section, may be regarded as radii of the fascicle circle.

Observations taken on a great number of fascicle cross-sections indicate that the above assumptions are valid, unless the fascicle has undergone dehydration or has been treated for sectioning.

To calculate volumes of individual fascicles it is necessary that lengths and average diameters be determined. In order to obtain diameter measure-

ments at equidistant intervals it is necessary to bring the needles of a fascicle together in proper orientation with radial walls in contact. A satisfactory practice is one in which thread ligatures, each with a single overhand knot, are slipped on the fascicle from its sheath end. All the knots are then tightened while the ligatures are located closely together just above the fascicle sheath. In this region the needles are not twisted and the radial surfaces are contiguous. The ligatures are slipped downward toward the apex and one is placed just above or below each point of diameter measurement. Twisted needles are in this way forced into a corrected position in the fascicle. With the ligatures holding the needles together the desired diameters may be easily taken with a microcaliper.

If, then, the actively transpiring surface, that containing the stomates, of an essentially 3-needled pine, such as loblolly pine (*Pinus taeda* L.), includes the external surface area of the fascicle, together with the surface of the 6 radial faces, calculation of surface area and volume of the fascicle is easily performed. Suppose, for example, that diameter measurements, d_1, d_2, d_3, d_4, and d, are taken at equidistant intervals along the fascicle from base to tip. Since d_5 represents the tip diameter its value, obviously, is zero. Consequently if the length of the fascicle be denoted by l, then the outer surface, o, of the fascicle may be represented

$$o = (\pi(l)/5) [d_1 + d_2 + d_3 + d_4]$$

while the inner surface, i, may be expressed

$$i = (3(l)/5) [d_1 + d_2 + d_3 + d_4]$$

Hence the entire transpiring surface, y_L, of a loblolly pine fascicle is the sum of these components, that is

$$y_l = o + i = (l/5)(d_1 + d_2, + d_3, + d_4)(\pi + 3) \tag{1}$$

The volume, x, of the same fascicle may be derived as readily. Since volume is the product of length, l, and the average cross-sectional area, it follows that

$$x = (l/5)(d_1^2 + d_2^2 + d_3^2 + d_4^2)(\pi/4) \tag{2}$$

By contrast, the expression for the transpiring surface of a particular 5-needled pine, such as eastern white pine, (*Pinus strobus* L.), differs from equation (1) in that stomates occur only on the radial faces of the needles of a fascicle. In this discussion it is assumed that transpiration from non-stomated surfaces is negligible and these surfaces are therefore neglected. In this case, then, o is zero, and the entire transpiring surface, y_W, may be represented

$$y_W = l (d_1 + d_2 + d_3 + d_4) \tag{1a}$$

The expression of fascicle volume of a 5-needled pine is, of course, identical with that of equation (2).

The transpiring surface and the volume of each fascicle of a random sample of 25 seedling loblolly pine needle fascicles were calculated according to equations (1) and (2), respectively, after taking the fascicle diameter measurements d_1, d_2, d_3, and d_4, in hundredth millimeters, and fascicle

length, l, in millimeters according to the above observational program. Upon plotting surface, y_L, on volume, x_L, of the individual fascicles in a system of rectangular coordinates, as in fig. 1 (upper graph), it was surprising to
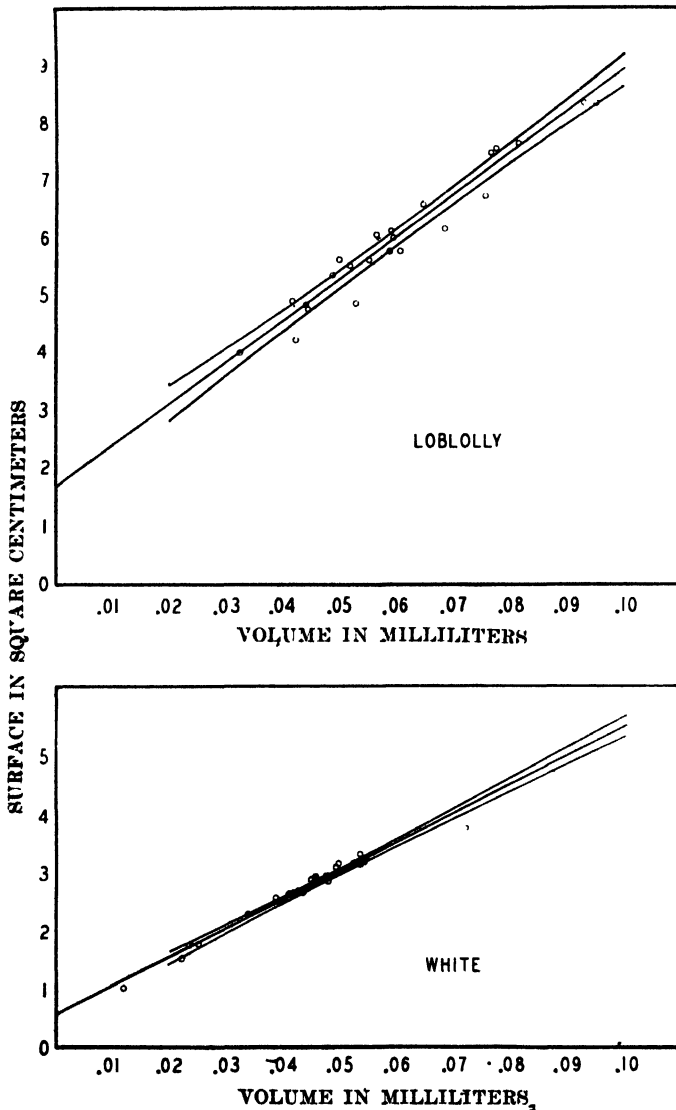

FIG. 1. Regressions and confidence bands of surface on volume of fascicles of loblolly pine and white pine. Based on data from 25 fascicles selected at random from each species.

find that the relation between the variables is not of a complex nature as had been anticipated; for it may, evidently, be adequately expressed by a straight line fitted to the plotted points.

Corresponding measurements were taken on a random sample of 25 fascicles of seedling eastern white pine needles using equations (1a) and

(2), respectively, in calculating surface and volume. Again, the discrepancies of the plotted points from the straight line fitted thereto (fig. 1, lower graph) substantiate the adequacy of the straight line.

For determining aggregate volumes of large groups of fascicles the immersion method was employed. Several techniques were investigated and it was found that methods in which overflow water was weighed are not very suitable in this work. In immersing an aggregate of fascicles in water air bubbles are often trapped in the network as it is placed in the vessel. A suitable method is one in which graduated cylinders are used and not completely filled before immersion. Trapped air bubbles may be released by pressing a bundle to the bottom of the cylinder with a glass rod.

The outline of the scheme of calculating the entire transpiring surface of any larger group of fascicles now becomes apparent. If, for instance, a 3-year-old loblolly pine has an aggregate of 1,000 fascicles, and the volume of the aggregate, as determined by immersion and the measurement of displacement, is 55.0 ml., one may enter the upper graph of figure 1 with the average volume to the fascicle (in this case, 0.055 ml.) and read therefrom the corresponding average surface (in this case, about 5.6 sq. cm.). Thus the entire transpiring surface of the 1,000 fascicles is about 5,600 sq. cm.

The experiment on transpiration rates which suggested the present problem required a high degree of precision of the calculated transpiring surface, as the transpiration rate per *unit* of transpiring surface of loblolly pine was to be compared with the corresponding rate of white pine under laboratory conditions. To this end a straight line of the form

$$y = a + b \ (x - \bar{x})$$

was fitted to the plotted points of figure 1, according to the method of least squares of FISHER (1) separately for each species. In this expression, a is the arithmetic mean in square centimeters of the 25 surface calculations of the sample for one of the species, and $\bar{x}$ is the mean in cubic centimeters of the corresponding volume calculations; while b is the computed rate of change of surface to the unit change of volume.

Upon applying the method to the sample of 25 loblolly pine fascicles as a case illustration, the expression of surface in terms of volume was found to be

$$y_L = 6.078 + 70.880 \ (x - 0.0621) \tag{3}$$

and the square of the standard deviation of the discrepancies between observed y_L and corresponding y_L is

$$s_L^2 = 0.07785$$

in (sq. cm.)^2. The estimate of the standard error of any calculated Y_L is the square root of

$$0.07785 \ [1/25 + (x - 0.0621)^2/0.007452] \tag{4}$$

The straight line in the upper graph of figure 1 is the representation of equation (3) for loblolly pine. The band which this line bisects throughout its length is known as the 95 per cent. confidence band. Its vertical distance (in positive and negative direction) from the bisecting line is twice the

standard error of the calculated Y_L as given by equation (4). It thus delimits the range which contains the true surface area, corresponding to given fascicle volume, with a probability of 0.95.

If equation (3) is now applied to the 1,000 fascicles, the mean volume of which, as used in the illustration above, is 0.0550 ml., the calculated average transpiring surface is the following:

$$Y_L = 6.078 + 70.880\,(0.0550 - 0.0621)$$
$$= 5.575 \text{ sq. cm.}$$

The estimate of the standard error of this value is found upon inserting 0.0550 for x in equation (4); whence the standard error of 5.575 is estimated to be the square root of 0.003641, or 0.0603 in square centimeters; and the entire transpiring surface area of the thousand fascicles is

$$1,000\,(5.575 \pm 0.0603) = 5,575 \pm 60.3$$

in sq. cm. If the standard error be doubled it expresses the 95 per cent. confidence interval. Hence, with probability of 0.95, the true aggregate surface of the thousand fascicles lies within the range

$$5,575 \pm 121$$

sq. cm. or within 2.2 per cent. of the calculated value. The estimate thus possesses quite a satisfactory precision, derived as it is, from careful measurements taken on only 25 fascicles. It is, of course, subject to unknown systematic error if the assumptions upon which the calculations of surface and volume of the 25 fascicles had been based, are not valid.

Summary

1. The surface area of a population of pine needle fascicles may be readily calculated through its correlation with volume, a value more easily determined by displacement.

2. A linear relationship of the form

$$y = a + b\,(x - \bar{x})$$

was found for needle fascicles of loblolly pine and eastern white pine. In the above expression, a is the arithmetic mean in square centimeters of all surface calculations of the sample for one of the species, and $\bar{x}$ is the mean in milliliters of the corresponding volume calculations; while b is the computed rate of change of surface to the unit change of volume.

3. The surface area of a population of fascicles may be readily determined by substituting in the equation above and determining the surface area of the average needle fascicle. By multiplying this value by the number of fascicles in the population the total surface area is easily obtained.

4. In establishing the graphic relationship between surface and volume of needle fascicles from a sample a microcaliper may be used to obtain diameter measurements along fascicles.

5. Thread ligatures are suggested as aids in obtaining diameter measurements.

This study was carried out at the School of Forestry, Duke University, and the writers are indebted to Dr. PAUL J. KRAMER of that institution for critical aid in the preparation of the present contribution.

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LITERATURE CITED

1. FISHER, R. A. Statistical methods for research workers. Oliver and Boyd, London. 1936.
2. GROOM, P. Remarks on the oecology of coniferae. Ann. Bot. **24**: 241–269. 1910.
3. HÖHNEL, F. VON. Über die Transpirationgrössen der forstlichen Holzgewächse. Mittheil. förstl. Versuchwesen Oesterr. **2**(1) : 47. 1879.
4. ——————. Weitere Untersuchungen über die Transpirationgrössen der forstlichen Holzgewächse. Mittheil. förstl. Versuchwesen Oesterr. **2**(3) : 1880.
5. KRAMER, P. J. An improved photoelectric apparatus for measuring leaf areas. Amer. Jour. Bot. **24**: 375–376. 1937.
6. MINCKLER, L. S. A new method for measuring transpiration. Jour. Forestry **34**: 36–39. 1936.
7. NEWMAN, S. A. The grid area method for determining areas of irregular figures; an application of the mean ordinate principle. Jour. Forestry **38**: 632–635. 1940.
8. UHL, ANTON. Untersuchungen über die Assimilationverhältnisse und die Ursachen ihrer Unterschiede in der Gattung *Pinus*. Jahrb. wiss. Bot. **85**: 368–421. 1937.

A TECHNIQUE FOR TREATING SMALL SEEDLINGS
WITH COLCHICINE

Ross C. Thompson

(WITH TWO FIGURES)

Numerous techniques have been employed by various workers in attempts to induce polyploidy in plants by the use of colchicine. No one procedure appears to be the best for all kinds of material, because the technique must be varied to meet the requirements of the particular material and conditions.

There is fairly general agreement that the colchicine treatment of germinating seeds and very small seedlings is more likely to result in polyploids than the treatment of growing points of older plant organs. The writer has found this to be especially true in the treatment of several species of Lactuca. Several methods of applying colchicine to the growing points of large plants, as in lanolin paste or water-glycerine solution by immersion or with saturated cotton, have given poor results. These methods have all resulted in deformed growing points with varying amounts of polyploid and diploid tissues. In nearly every case, however, where lateral buds were treated, the tissues returned largely to the diploid form, and seeds containing only embryos having the diploid number of chromosomes resulted. In contrast to the high percentage of failures resulting from the treatment of growing points on older plants, the number of polyploids obtained from the treatment of germinating seeds and small seedlings has been quite high. However, neither the germination of Lactuca seed in weak concentrations of colchicine nor the soaking of seed in colchicine solutions previous to germination has proved entirely satisfactory. While a fairly high percentage of tetraploids has been obtained from both of these methods they have resulted in extreme retardation of radicle and root development with a consequent loss of many of the seedlings before they developed sufficient root systems to continue growth.

A method of treatment has been worked out whereby the growing apex of the seedling can be treated without subjecting the radicle and rootlets to the deleterious effect of the chemical. The procedure permits the treatment of a large number of small seedlings with a limited amount of labor. For successful application it is necessary that the seedlings have relatively long hypocotyls. The length of the hypocotyl in many seedling plants can be controlled to some extent by conditions just following the breaking of the seed coat in the process of germination, and before treating with colchicine.

In Lactuca seedlings the length of the hypocotyl has been greatly increased over normal by placing the germinating seeds in darkness on a moist surface at a temperature favorable for rapid cell activity (25°–30° C.) at

the time the radicles appear through the seed coats. In the absence of light the hypocotyls become much elongated and etiolated. When the elongation has reached its maximum, or about 20 mm., the seedlings are ready to be given the colchicine treatment. The conditions required for forcing hypocotyl elongation and etiolation may have to be altered somewhat for different kinds of material, and for some species the procedure is not at all practicable; however, it can be used to good advantage with seedlings of a great many species.

Materials and methods

A Petri dish and a disc of paper about one centimeter larger in diameter than the dish are required for each treatment. The paper can be prepared easily by folding a filter paper, in the manner indicated in figure 1, and
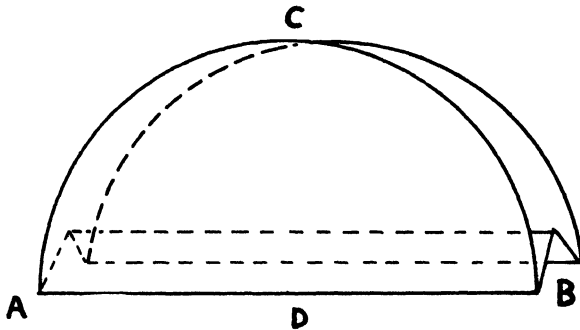

FIG. 1. A filter paper properly folded for cutting the disc to fit the bottom of a Petri dish. The distance A–B should be the same as the diameter of the bottom of the dish. The distance from C to D should be slightly less than one half of A–B.

from this cutting a semi-circular piece about equal to one-half of the bottom of the dish. The paper is then placed in the dish as shown in figure 2 and sealed in place with melted paraffin. The dish may be slightly warmed to
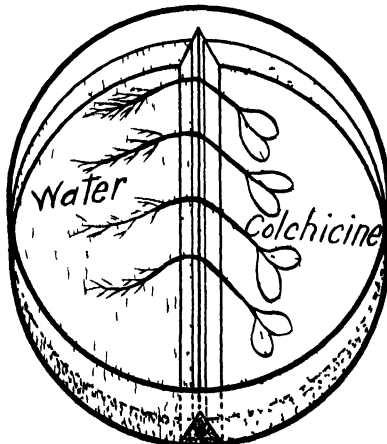

FIG. 2. A Petri dish equipped for the treatment of small seedlings with colchicine solution.

prevent too rapid cooling of the wax. With the paper held in place a small amount of hot paraffin is poured into the dish while it is held in an inclined position and so that the paraffin is made to flow around the margin of the paper, sealing it to the bottom of the dish. After a few rotations the paraffin will be cooled sufficiently to hold the paper in place. A little more warm paraffin may be applied at the ends of the ridge in the paper so as to seal it to the dish at both ends. The apex of the ridge should be greased with a little vaseline or some other non-injurious grease or oil to prevent the solutions, to be placed in the dish, from creeping over the ridge and becoming mixed; or the whole disc of paper may be treated with hot paraffin. The dish is now ready for use. Enough colchicine of the desired concentration is placed on one side of the ridge to come within about $\frac{1}{4}$ inch of the top of the ridge. The height of the ridge formed by the paper may be varied so as to provide a proper depth of the colchicine solution to cover the cotyledons and epicotyls of the seedlings being treated. The other half of the dish is filled with water. It is advisable in some cases to use a layer of damp cotton batting in place of the water for keeping the roots moist.

The elongated hypocotyls of the seedlings are placed across the ridge so that the roots are in the water or on the wet cotton, and the epicotyls in the colchicine solution. In some cases it has been found advisable to apply a little vaseline to the hypocotyls at the point where they cross the ridge. This can be done with a small camel's hair brush. If the hypocotyls are long it is not difficult to keep both ends of the plant submerged. If the hypocotyls are short it may be necessary to weight the ends down lightly to keep them submerged. The dishes may then be covered and left in a desirable light and temperature for the duration of the treatment.

The number of seedlings that may be treated in a dish at one time will depend on the size of the Petri dish used and the size of the seedlings.

After the seedlings have been exposed to the treatment for the desired time, usually 4 to 8 hours, they are removed and washed thoroughly in water, dried slightly on absorbent paper, and transplanted to soil where they are permitted to grow until the effect of the treatment can be determined. Because of the long hypocotyls of seedlings thus treated it is necessary that they be set deeper in the soil than is normal. Tetraploid stems on diploid roots obtained by the procedure outlined have been far more satisfactory than the types of tissues obtained by other methods so far tried.

A SIMPLIFIED INTEGRATING LIGHT RECORDER
FOR FIELD USE[1]

V. G. SPRAGUE AND E. M. WILLIAMS

(WITH TWO FIGURES)

In a previous paper (1) an inexpensive integrating light recorder was described which operated with current supplied from a transformer and rectifier connected to an ordinary alternating current line. This paper describes a similar unit in which batteries are used to supply current. In both instruments the minute current from a phototube charges a condenser producing a potential difference which gradually increases. When the potential across the condenser reaches the breakdown voltage of a cold cathode relay tube in parallel with the condenser plates, the gas in the tube ionizes discharging the condenser and at the same time discharging a larger condenser through a sensitive counter. A direct current supply is required to supply the phototube and provide the charge on the condenser which actuates the counter.

In order to make the integrating light recorder portable for use under field conditions, the original circuit was slightly modified to use dry batteries as a source of power. The circuit (fig. 1) is simplified by this change and the action of the counter is more positive. Since the current used in operation is very small (less than 10^{-6} amperes) very small batteries are adaptable and the useful life is approximately the shelf life of the batteries. The voltage required for operation of the phototube is reduced slightly (to 90 volts for convenience in tapping when 45-volt batteries are used), while the voltage required for operation of the counter is reduced considerably, from 280 volts in the alternating current instrument to 135 volts when batteries are used. The 250,000-ohm resistor across the condenser (C_2) used to actuate the counter in the alternating current instrument is not required since the batteries can be tapped directly for the required voltage. It has been found that an 0.5-mfd. high grade paper condenser is approximately the correct capacity for condenser C_1 and that a 2-mfd. paper condenser is usually satisfactory for condenser C_2. A 250,000-ohm resistor is placed in series with the + 135-volt terminal of the battery and condenser C_2 to prevent continuous ionization of the gap between the control anode P_2 and the cathode K of the relay tube.

A receiver, similar in design to that described by WALLACE (2), has been used for two years and has been found to be quite satisfactory. A spherical globe (fig. 2) is countersunk into a wooden block through which a square hole has been cut immediately below the globe, approximately the same size

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[1] Contribution no. 41, of the U. S. Regional Pasture Research Laboratory, Division of Forage Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, in cooperation with the northeastern states and the Electrical Engineering Department of The Pennsylvania State College, State College, Pennsylvania.
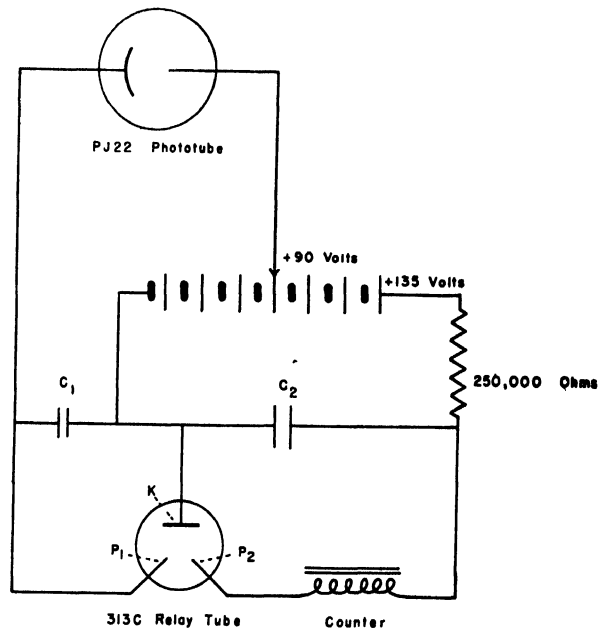
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FIG. 1. Wiring diagram of battery operated integrating light recorder.

as the opening in bottom of the globe. The phototube is mounted in a light-tight box which is fastened to the under side of the wooden base. The receiving surface of the phototube faces the globe. Inasmuch as the maximum incident intensity on the phototube should not exceed 500 foot candles, it is necessary to reduce the received intensity by placing a piece of frosted opal glass at the base of the globe and at the top of the square hole. This acts as a receiver for the light transmitted by the globe and in turn as a transmitter of light to the phototube directly beneath it. With this arrangement

FIG. 2. Battery operated integrating light recorder and the opal glass spherical receiver.

the light reaching the phototube rarely exceeds 450 foot candles. A lead-covered cable provides a satisfactory weatherproof lead-in from the receiver to the recorder.

In calibrating the instrument a clear day without clouds is most satisfactory. A heavy paper cylinder at least four feet long and just the diameter of the globe is used. One end of the cylinder is placed over the globe and the other is pointed directly at the sun so that only parallel light rays strike the receiver. The base and other portions of the globe are covered with dark cloth to prevent other light from striking the globe. With a stop watch, the number of discharges per minute is determined and immediately thereafter the tube is removed from the globe and the intensity of the light at the base of the paper cylinder is read with a standardized foot-candle meter. Several readings should be taken which may then be used in calculating the calibration factor for the globe, phototube, and instrument. Since phototubes vary slightly in sensitivity, it is necessary to obtain a new calibration factor if the phototube is changed. Although phototubes have nearly constant sensitivity, it is probably desirable to recalibrate the instrument every six months and to remove any dirt or dust which may have accumulated on the globe, on the frosted opal glass plate, or on the phototube. If the recorder is used under field conditions, it is advisable to place everything but the receiver under cover. It should be subjected, however, to free circulation of air, since excessive ambient temperatures result in decreased battery life. Such stations as those used by the Weather Bureau to determine maximum and minimum temperatures and humidities are satisfactory. Inasmuch as this recorder develops no heat during operation, it may be thoroughly enclosed to keep dust and blowing rain from reaching the instrument. The total cost of the parts for this instrument was approximately $12.00.

It is probable that an alternating current instrument is preferable where continuous records for a number of years are desired and where the recorder can be placed in the laboratory; but for field operation, or wherever a portable recorder is required, the battery operated set is especially adaptable.

U. S. REGIONAL PASTURE RESEARCH LABORATORY
AND
THE PENNSYLVANIA STATE COLLEGE

LITERATURE CITED

1. SPRAGUE, V. G., and WILLIAMS, E. M. An inexpensive light recorder. Plant Physiol. **16**: 629–635. 1941.
2. WALLACE, R. H. Methods of sampling visible radiation. Plant Physiol. **12**: 647–666. 1937.

A LIGHT–TIGHT BOX FOR MAKING SHADOWGRAPHS OF AVENA SEEDLINGS FOR GROWTH HORMONE DETERMINATIONS

R. E. Bennett, S. A. Gordon, and S. G. Wildman

(WITH ONE FIGURE)

The authors have made and are using a simple light-tight box for making shadowgraphs of Avena, quickly and easily, without leaving the constant-temperature-constant-humidity culture room. After visiting other laboratories, and talking to other workers it is our impression that usually the rows of seedlings are taken out of the culture room to an adjacent dark room for making the exposures. To shadowgraph 38 rows requires opening the door 76 times, allowing large volumes of dry cool air to enter the culture chamber. Tests with a hygro-thermograph showed that the humidity varied more than 20 per cent. during the interval that shadowgraphs were being made, whereas at other times it remained nearly constant. A further disadvantage of carrying the seedlings to another room is the danger that the coleoptiles may be bent or disarranged while opening and closing a heavy door such as is used for a culture room.

By experimentation it was determined that the distance between the light and the seedlings could be decreased to less than six feet without introducing any appreciable error from parallax. Sharply defined shadows were obtained by using a clear (non-frosted) bulb. Even more sharply defined shadows were obtained with a six-volt automobile tail-light bulb for illumination.

The essential features of the box which was designed and built are shown in the accompanying figure. Since the box is used inside the culture room under conditions of high humidity, it is suggested that waterproof plywood be used in construction to minimize warping. All joints should be glued with a resin glue such as "Cascamite" or "Weldwood." White light is confined to the interior of the box by the use of baffles on the door, instead of felt, because a slight warping or a poor fit would allow light to leak around felt. A ruby bulb is placed on the outside and is wired in series with the clear (non-frosted) 40-watt bulb which is inside the box. The ruby bulb serves as a pilot light and also reduces the voltage on the white bulb so that exposures with "Kodabrom" contrast bromide paper are about four seconds. A switch is placed on the cord rather than on the box, to minimize vibration during exposure. A small ruby bulb (not shown in the figure) is placed inside the box to facilitate handling the seedlings and the photographic paper. This bulb is connected directly to the line so that it remains burning all of the time. .

The use of this box in the culture room has considerably speeded and facilitated the shadowgraphing of Avena seedlings. It is small enough to cause no inconvenience in the culture room. No white light escapes from the box. In this laboratory two persons work together and use as many as

38 rows of seedlings for one experiment. While one person is finishing the placing of agar blocks the other can begin shadowgraphing, with confidence
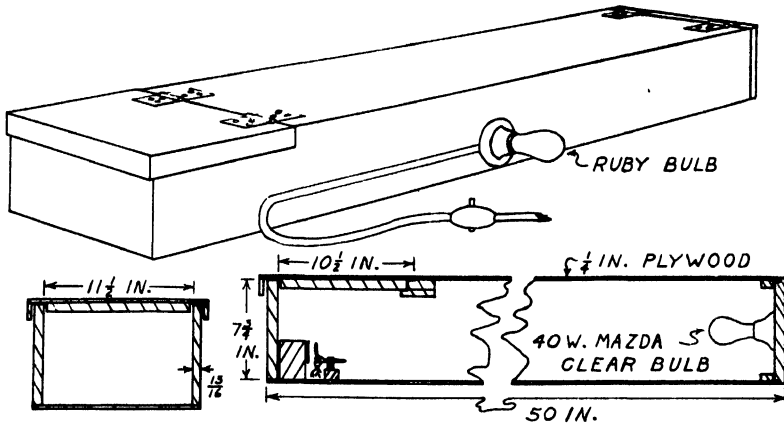

FIG. 1. Above, a light-tight box for making shadowgraphs of Avena seedlings; below, left, cross section of the box showing construction of the door; right, median longitudinal section of the box showing placement of the paper holder, seedling holder, and the white light.

that the last part of the experiment will not be altered by humidity or temperature changes.

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    ANN ARBOR, MICHIGAN

NOTES

Meeting Canceled.—At the request of the Office of Defense Transportation the meetings which were to have been held in New York late in December, 1942, were postponed or canceled in order to relieve the congestion of army holiday travel. In most respects we can go along about as well without the meetings; but it does make it more difficult to have intelligent consideration of the business matters which should have received attention at the annual meeting. It is hoped that the Executive Committee will not consider any matter settled until the reasons for proposed actions can be thoroughly understood, and appropriately digested. The main committee reports will be presented in this number of PLANT PHYSIOLOGY.

Life Membership Award.—The CHARLES REID BARNES life membership committee reported the selection of Dr. OLENUS LEE SPONSLER, Professor of Botany at the University of California at Los Angeles, to receive the nineteenth award of this honor. The award is made in recognition of Dr. SPONSLER's contributions to the knowledge of molecular structure of biological materials as revealed by x-ray methods. Dr. SPONSLER is a native Ohioan, with degrees from Michigan, Nebraska, and Stanford. He has been connected with the University of California since 1922. The Society is greatly honored by the addition of Dr. SPONSLER to its roll of CHARLES REID BARNES life members.

Hales Award.—The eighth award of the STEPHEN HALES prize has been made to Dr. CORNELIS BERNARDUS VAN NIEL, Professor of Microbiology at the Hopkins Marine Station, Stanford University, in consideration of his brilliant work on the biochemistry of lower organisms, and particularly his contributions to an understanding of photosynthesis among these organisms. Dr. VAN NIEL was born at Haarlem, Holland, November 4, 1897. He was trained at the Technical University at Delft, where he obtained his degrees, and where he served as conservator of the microbiological laboratory from 1924 to 1928. In the latter year he was attached to the Hopkins Station, where he has worked for 14 years.

His best known work deals with bacterial photosynthesis. When he first began to study the purple and green sulphur bacteria (*ca.* 1926) knowledge of the nutrition and metabolism of these organisms was fragmentary and contradictory. Several theories had been advanced, some believing that photosynthesis was, in these forms, not much different than in green plants; others believed them to be chemosynthetic; still others thought the metabolism was a mixed chemosynthetic photosynthetic process. The difficulty was to reconcile the following observations:

1. Light and oxidizable sulphur compounds are required.
2. No oxygen is liberated.

3. The sulphur compounds are oxidized generally to sulphates in the absence of oxygen.
4. Carbon dioxide is consumed.

Dr. VAN NIEL (1930–1931) clarified the problem by a combined experimental and theoretical approach. He isolated (for the first time) pure cultures of purple and green sulphur bacteria, studied the influences of light, oxygen, oxidizable sulphur compounds, carbon dioxide, etc., on their growth, and established the quantitative relations among the substances consumed (CO_2, H_2S) and produced (SO_4, cell material) in the light of metabolism. The experimental results were interpreted in terms of a new type of photosynthesis which, when H_2S is the oxidizable substrate and sulphur the oxidation product, is expressed by the equation:

$$CO_2 + 2H_2S \rightarrow (HCHO) + H_2O + 2S$$

generalized, thus: $CO_2 + 2H_2A \rightarrow (HCHO) + H_2O + 2A$,

where H_2A is any oxidizable substrate, organic or inorganic.

These bacterial photosyntheses are sharply distinguished from green plant photosynthesis by their dependence upon the presence of an oxidizable substrate other than water, and by the absence of any oxygen production.

VAN NIEL's generalized theory of photosynthesis has had an important influence upon subsequent experimental studies of green plant photosynthesis; witness the work of GAFFRON on photosynthetic hydrogen utilization by Scenedesmus, and RUBEN's demonstration by the use of isotopes, that the oxygen of photosynthesis is derived from water, not from carbon dioxide.

In addition to these studies of photosynthesis, VAN NIEL has contributed important papers on the morphology, nutrition, taxonomy and biochemistry of propionic acid bacteria; and his work on the fermentation of glycerol provided an essential link in the chain of events which led to the discovery of the rôle of carbon dioxide in the formation of succinic acid and other products by WOOD and WERKMAN in 1936. He has also made important contributions to bacterial taxonomy in general, in collaboration with KLUYVER and STAVIER.

Other subjects which have claimed his attention are methane fermentation, fermentative assimilation, diacetyl and butter aroma, and a new genus of yeast-like organisms, Sporobolomyes.

Not the least of his accomplishments is his ability as a teacher. An actor at heart, he has the rare gift of being at the same time precise, logical, and inspirational. These and other qualities make him the idol of his students, the wonder and delight of his friends.

The committee on the award, made up of the last three recipients of the award, have made a splendid selection, one which merits universal approval. It accords with the spirit of the Society, and with the spirit of those who, in 1927 and later years, by their gifts, laid the foundations for this biennial award. We can all be justly proud that the honor has been bestowed on one so well deserving it.

Editorial Report.—In reporting the activities of the editorial committee for 1942, attention was directed to several matters of general interest. The volume for 1942 showed considerable reduction in size from that of 1941, a total of 717 pages as compared to 857 in 1941. The reduction came about without sacrifice, because the demands for space were smaller in 1942 than in 1941. For instance, the 66 papers published in 1941, averaged 13 pages each; whereas the 70 papers published in 1942 averaged only 10 pages each. The decrease in size of volume 17 is, therefore, an expression of the more concentrated and less verbose type of paper which has been coming into favor among scientists generally. This is an encouraging trend, and, if it is maintained, should cushion the impact of war upon our finances. Authors should keep in mind at all times that care in the preparation of manuscripts will dispense with unnecessary repetitions, will not present or stress the obvious and well-known facts, and will reduce summarizing of past literature to the minimum requirements. The editors appreciate the progress investigators are making in the direction of succinct reports, but desire to retain our practice of giving space to lengthy papers when more space is needed.

George Peter Hoffman.—On April 1, 1941, GEORGE PETER HOFFMAN, who was connected with the U.S.D.A. horticultural field station at Meridian, Mississippi, died of a heart ailment. He was born in Columbia, South Carolina, February 23, 1890. His advanced education was obtained at Clemson College, where he was graduated in 1915; and at Cornell University (M.S., 1925), and Michigan State College, 1932. He was an assistant in horticulture at Clemson in 1916, went into extension work with them for about six years, first in general extension work and then as extension horticulturist until 1923. He then entered the Department of Horticulture as associate professor, a position which he filled for three years. In 1926 he went with Penney Farms, the J. C. Penney-Gwinn Corporation, which he served for six years as manager of its program. In 1932 he was appointed to a position in the Department of Agriculture in nut investigations, and was soon stationed at Meridian, where his work was mainly concerned with pecans, grapes, sweet potatoes, and vegetables.

He is survived by his wife, who lives at Fountain Inn, South Carolina, and a son who is in attendance at Clemson College.

Fellowship.—Sigma Delta Epsilon continues its award of a $1000 fellowship. The second award is to be made for 1943–1944, and applications and references should be submitted before March 1, 1943. Women with the equivalent of the master's degree, with research in mathematical, physical, or biological sciences, who need aid to complete their work, are eligible. They must give evidence of high ability and promise in research. Those receiving the award are supposed to devote the major time to research on some approved subject, and not to do work for remuneration outside the fellowship, unless such program has been specifically authorized and approved.

Application blanks may be obtained from Dr. ELOISE GERRY, care of the U. S. Forest Products Laboratory, Madison, Wisconsin. Interested persons are invited to write, and to mark correspondence "Personal."

Sigma Delta Epsilon may well be proud of its determination to carry on this valuable work during these desperate times.

Selective Service.—While the rules for selective service and selective deferment are so much subject to change that statements do not remain accurate for very long, the following notes are culled from a statement on occupational deferment issued under date of September 30, 1942.

The War Manpower Commission has certified that educational services are essential to the support of the war effort. Under such services the Commission specifies public and private industrial vocational training; elementary, secondary, and preparatory schools; junior colleges; colleges, universities, and professional schools; educational and scientific research agencies; and the production of technical and vocational training films.

A list of critical occupations is given in occupational bulletin 23, and among others the following are of interest to our membership: Agricultural sciences, bacteriology, biology, chemistry, and physiology. In general, occupations requiring more than 6 months of technical training are to receive consideration at the hands of all draft boards. Full time instructors in biology in elementary schools, and presidents, deans, and registrars in junior colleges, professors and instructors engaged in full-time instruction in the subjects mentioned, are considered as engaged in work critically related to the war effort.

This information is given because our members may not be aware of the privileged status of their work as full time instructors in critical occupations.

Carnivorous Plants.—One of the most interesting and romantic chapters in plant physiology is that which deals with the carnivorous plants. Those who have been privileged to read the account in KERNER and OLIVER's *Natural History of Plants* which deals with these interesting creatures, will turn to a new account of them with expectant enthusiasm. For more than a decade Dr. FRANCIS E. LLOYD has been studying these plants intensively, and has now brought his observations into book form. It is a handsome monograph entitled *The Carnivorous Plants.* It is published by the Chronica Bontanica Company, of Waltham, Massachusetts.

The introductory chapter gives a general account of these curious plants, their geographic distribution, the types of traps and pitfalls that have been developed, and the difficulties of accounting for them on any evolutionary hypothesis. The reviewer has always wished to hear some survival-of-the-fittest philosopher set himself to the task presented by these complicated trapping devices.

The succeeding chapters take up genera or species for detailed consideration, and the chapters bear the following titles: Heliomphora; Sarracenia; *Darlingtonia californica;* Nepenthes; *Cephalotus follicularis;* Genlisea; By-

blis; *Drosophyllum lusitanicum;* Pinguicula; Drosera; carnivorous fungi; Dionaea; Aldrovanda; Utricularia, Biovularia and Polypompholyx; and the Utricularia trap.

The illustrations occupy 38 plates at the close of the text, and these drawings help to make the text descriptions clear. It is a delightfully written account, and summarizes as well as can be done at present, the construction and operation of the trapping devices described. One can well imagine that the author of the work found the study most fascinating, and the work as a whole is a monument to Prof. LLOYD's industry and scholarship.

The book is volume 9 in a series edited by FRANS VERDOORN. The price of the book is $6.00 per copy. Orders may be sent to Waltham, Mass., The Chronica Botanica Co., or New York City, G. E. Stechert and Co., the publishers.

Autonomic Regulations.—A book of interest to premedical and medical students, and of physiologists by Dr. ERNST GELLHORN, of the College of Medicine of the University of Illinois, deals with autonomic regulations in the animal body. The general introduction presents the anatomical and physiological foundations of the subject.

The second section deals with adjustment reactions involving primarily the respiratory and circulatory systems, such as adjustment reactions to carbon dioxide, anoxia, asphyxia, hemorrhage, hypoglycemia, and regulation of cerebral circulation.

Part III discusses autonomic-endocrine integration; part IV, autonomic somatic integration; and part V with applications, and the physiological and clinical aspects of autonomic regulations.

Although less valuable to plant physiologists than to animal and human physiologists, it should stimulate thinking in comparative terms of plant and animal reactions. It is a book of 373 pages, and is published by Interscience Publishers, Inc., 215 Fourth Ave., New York. The price is $5.50 per copy.

Immunology.—A book on fundamentals of immunology, written by Dr. WILLIAM C. BOYD, of the Boston University School of Medicine, surveys the information in this field. It contains eleven chapters, as follows: Immunity and immunology; antibodies and antibody specificity; antigens; cell antigens; blood groups; antibody-antigen reactions; complement and complement fixation; anaphylaxis and allergy; allergy and immunity, bacteria, viruses, parasites; practical use of artificial immunity; and laboratory and clinical techniques.

Certain phases of this work should be of particular interest to plant pathologists, and virus investigators. It is intended primarily for the student of medicine and animal biology, but again has value in comparative physiology and pathology of animal and plant life.

It contains 446 pages, and is quoted at $5.50 per copy. The publishers are Interscience Publishers, Inc., New York. Biological libraries should be expected to make works of this type available to all biological investigators.

PLANT PHYSIOLOGY

APRIL, 1943

A SYSTEM OF ANALYSIS FOR PLANT TISSUE BY USE OF PLANT JUICE[1]

FRANK S. SCHLENKER

The utilization of expressed plant juice for determining the chemical constituents of a plant is becoming increasingly important. It has the advantage of presenting the products of metabolism in a state more similar to that found in living tissue than any of the more commonly used extraction methods. Conditions of state and equilibrium, however, can probably never be identical for juice within and without the cell, for at the moment of cell rupture, the conditions that control living cellular activity are changed. To illustrate, if living leaf tissue is placed in an atmosphere of carbon dioxide, the juice becomes more basic (8), but if the cell liquid is expressed, and carbon dioxide bubbled through it, the juice becomes more acid.

The first important studies of expressed juice were for the investigation of physical constants such as density, refractive index, freezing point, etc. This work has been summarized by SASTRI and SREENIVASAYA (21). Recently, attention has been paid to the inorganic and more particularly to the organic fractions, of plant juice including the sugars and nitrogen fractions. This paper is presented to describe methods for the determinations of a portion of the inorganic and organic constituents in plant juice extracts, and calculation of the results on a fresh weight basis.

General methods

Before a representative sample of juice can be obtained from the entire tissue studied, the plant material must be treated in some manner so that the contents of all cells are represented. For example, unrepresentative juice samples are obtained by pressure alone, if the freezing point lowering is used as a criterion. This uncertainty may be obviated, to a large extent, by treating the sample in various ways: by grinding, freezing with solid carbon dioxide, liquid air, or eutectic mixtures; by vapors of volatile liquids such as ether or chloroform; or by autoclaving (1, 6, 7, 11, 12, 21). The work of SAYRE and MORRIS (22) with corn indicates that grinding is as effective in breaking cell walls as freezing. This is advantageous for there

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is some indication that during the freezing of tomato leaves the sucrose-reducing sugar equilibrium is shifted toward an increase in reducing sugars. Substances such as nitrates, inorganic phosphates, and total sugars are expressed in like amounts in successive fractions, after a preliminary grinding of corn tissue (23). Treatment of plant material by autoclaving at five pounds per square inch for 15 minutes gives results comparable to the freezing technique (7) and similarly causes the precipitation of proteins.

For the data presented in this paper, the ordinary Nixtamal mill was used for grinding leaf samples. When samples were frozen, they were first cut and wrapped tightly in a cheese-cloth bag, exposed to carbon dioxide snow as it was formed at the end of a copper tube attached to the tank of liquid carbon dioxide. This gave rapid and complete freezing of a 100-gram sample in less than a minute. Unless otherwise stated, all frozen samples used in this study were so treated.

It is evident that the preliminary treatment of plant tissue depends upon the substances to be determined. Grinding is apparently adequate for all circumstances and its only evident disadvantage, in comparison with freezing or autoclaving, is that the resulting juice contains large quantities of debris and protein. Where proteins must be removed, their partial elimination by freezing or autoclaving is helpful.

DETERMINATION OF PHYSICAL CONSTANTS

SAYRE and MORRIS (23) have proposed a formula[2] whereby the various plant constituents can be determined by analysis of expressed juice and the results calculated to the fresh-weight basis. The values obtained were comparable to those secured by an alcohol extraction. Using this equation as a basis, it was advisable to examine the methods available for the determination of specific gravity, total solids of plant juice, and the total moisture of the leaf material since these values are necessary for the calculation.

For the determination of specific gravity three methods are available: the Westphal balance, hydrometer, and pycnometer. In a series of 20 juice samples of beet and tomato leaves using the methods mentioned, the average values obtained were 1.036, 1.025, 1.022, respectively. In all instances the agreement between these methods was sufficiently accurate so that any one could be used. The hydrometer method, however. is by far the most rapid and convenient.

GORTNER (13) has demonstrated the possibility of determining total solids and subsequently the moisture content of plant juice with the refractometer. This method has been shown (17) to give high results in comparison with oven drying for juice samples of soybean, corn, wheat, and oats. SAYRE and MORRIS (23) found that for corn it was necessary to correct the total solids determined by refractometer when leaf juice was used, but no correction was needed for juice from stalk tissue.

$$\text{[2] Percentage of sugar in tissue} = \frac{\frac{\text{Gm. of sugar in}}{100 \text{ ml. sap}} \times \frac{\text{Total moisture}}{\text{of tissue in gm.}}}{\text{Gm. of moisture in } 100 \text{ ml. sap}}$$

In the present study a comparison has been made between the toluene distillation method (3) for moisture in plant juice and the refractometer method. The refractometer was adjusted to give the theoretical value for water at 20° C. The scale reading gives the refractive index of the juice directly, and SCHÖNROCK's (2) table was used to translate refractive index values to total solids. The percentage of juice moisture is the difference between the percentage of total solids and 100. In table I, the results by the above mentioned methods are expressed in terms of moisture content rather than total solids.

The estimation of total moisture in leaf tissue was also made by oven drying at 100° C. for 24 hours. In order to be assured of the accuracy of this method it was examined in two ways. First, samples were prepared by cutting the leaves into small sections and weighing aliquots into aluminum dishes. One series was frozen with carbon dioxide snow, the other was untreated. The freezing technique was introduced to make certain that the cell walls were broken so that all leaf moisture could be removed. Using juice from tomato leaves and beet leaves and beet petioles the agreement between frozen and non-frozen samples was within 0.5 per cent. Similarly, with the same tissues the average of six determinations gave the following results: oven drying, 86.88 per cent.; toluene distillation, 86.75 per cent. moisture.

CHEMICAL METHODS

In a study of the nitrogen metabolism of plants certain fractions are usually determined; nitrate, ammonia, alpha-amino, amide, and total nitrogen. Carbohydrate analyses usually include sucrose and reducing sugars, which in turn may be divided into glucose, fructose, and non-sugar reducing substances.

NITRATE NITROGEN.—BURRELL and PHILLIPS (4) for alcohol extracts and FREAR (10) for plant juice have shown that clarification of plant juice is possible with silver sulphate, copper sulphate, and a mixture of calcium

TABLE I

PERCENTAGE OF MOISTURE IN JUICE FROM BEET LEAVES AS DETERMINED BY REFRACTOMETER AND TOLUENE DISTILLATION METHODS

METHOD	MOISTURE, PERCENTAGE								
	1	2	3	4	5	6	7	8	AVERAGE
	%	%	%	%	%	%	%	%	%
Refractometer	91.93	89.93	93.20	93.90	95.20	93.90	94.50	95.20	93.46
Toluene distillation	92.00	91.40	92.90	94.50	95.00	93.00	93.40	93.90	93.26

hydroxide and magnesium carbonate or calcium hydroxide alone. BURRELL states that it was necessary to make a preliminary treatment with sodium peroxide for alcoholic extracts. FREAR did not try this for plant juice but secured satisfactory results. Nitrate is then determined in the clarified juice by the phenol-disulphonic acid method (15).

AMMONIA NITROGEN.—While studying the various methods for ammonia, SCHLENKER (24) found that when either 52 per cent. potassium carbonate or heavy magnesium oxide was added to plant juice and the sample aspirated, there was a rapid evolution of ammonia during the first two hours but that with continued aeration ammonia continued to be given off in small amounts over a long period of time. A more satisfactory procedure for the determination of ammonia was devised by shaking juice with 2.0 ml. of sodium permutit (FOLIN) for 5–7 minutes to adsorb the ammonia, then removing the juice by washing, thus eliminating the glutamine and asparagine. Ammonia is freed from the permutit by sodium hydroxide and aspirated as usual the Van Slyke-Cullen aspiration block.

PUCHER (19) later reported that the amide nitrogen of glutamine is hydrolyzed quite readily, more easily than that of asparagine. Therefore, it is to be expected that when glutamine is present in a plant juice which is treated with an alkali such as potassium carbonate and aspirated, some of the glutamine amide nitrogen will be included with that of the preformed ammonia. It can be shown that asparagine is only slightly hydrolyzed by potassium carbonate (27).

GERDEL (11) modified the SESSION and SHIVE method for the determination of ammonia and nitrate nitrogen; but, as this method is based on the release of ammonia directly from plant material in the presence of alkali, high results are obtained.

ALPHA-AMINO NITROGEN.—The VAN SLYKE (30) method for the estimation of amino nitrogen has been examined by STUART (29) who found that high results are obtained when using water or alcoholic plant extracts. A number of substances such as ammonia, dihydroxy phenols, and tannic acid were found to yield nitrogen with nitrous acid. Several means were used to eliminate these error-producing substances. The lowest amino nitrogen values were obtained after adding calcium oxide and using vacuum aspiration at 40–45° C. for one hour with subsequent removal of excess lime. It was further demonstrated that amino acid and asparagine nitrogen could be recovered quantitatively after this treatment. It is probable, however, that a portion, if not all, of the glutamine present was destroyed during the vacuum distillation.

Ninhydrin, a specific reagent for alpha-amino acids, has been used to determine the amino nitrogen in plant extracts (28). If the results using this reagent are taken as true values, the figures obtained by the nitrous acid method are too high even after treatment with lime. The reliability of the nitrous acid method depends on the extent to which interfering substances can be removed and apparently lime treatment is insufficient. In the use of ninhydrin it is necessary to precipitate proteins and desirable to remove preformed ammonia. Because of specificity, methods using ninhydrin will undoubtedly give results that are close to the true alpha-amino nitrogen content of plant extracts.

GLUTAMINE AND ASPARAGINE AMIDE NITROGEN.—These amides are usually determined together by hydrolysis with normal sulphuric acid but CHIBNALL

and WESTALL (5) and VICKERY *et al.* (31), by adjusting the hydrogen-ion concentration of the solution were able to hydrolyze glutamine in the presence of asparagine, with the inclusion of only a small amount of the latter. At pH 6.0–6.5 between 98.5 and 99.0 per cent. of glutamine amide nitrogen is hydrolyzed after two hours at 100° C. In the presence of normal sulphuric acid for three hours both amides are completely hydrolyzed. Asparagine amide nitrogen is obtained by difference on the assumption that only these two amides are present. This procedure has been applied to plant juice (27).

PUCHER (20) developed a method for the estimation of glutamine which supplements the method just described. This was based on the ethyl-acetate extraction of the pyrrolidone-carboxylic acid formed during the nearly neutral hydrolysis of glutamine. After extraction the carboxylic acid was hydrolyzed to glutamic acid. By determining amino nitrogen before and after the second hydrolysis the amount of glutamine present was calculated.

ORCUTT and WILSON (18) advocate the use of 20 per cent. sodium bisulphite to avoid humin formation which occurs during the determination of total amide by 10 per cent. sulphuric acid. It is probable that bisulphite may be used on juice samples to avoid the necessity of precipitating the protein fraction. Determinations of total amides were made on protein-free and protein-containing juice treated with 10 per cent. sulphuric acid and 20 per cent. sodium bisulphite (table II).

It is evident that bisulphite is sufficiently active to hydrolyze plant juice proteins so that it is necessary to remove these proteins before using this reagent; the same holds true for sulphuric acid.

DETERMINATION OF SUGARS.—The colorimetric method of FOLIN (9) using an alkaline copper tartrate oxidizing solution gives satisfactory results with plant juice (25, 26). In the preparation of the tissue extract, freezing is not always a satisfactory method, for in some instances during this process there is a shift in the sucrose-reducing sugar equilibrium with an increase in the reducing sugars. In tomato leaf reducing sugar increased 0.13–1.70 mg. per ml. of juice. This difference is not due to sampling errors for, when a sample of plant juice expressed from ground beet leaf was divided into two portions and only one portion frozen, its reducing sugar content was increased by 0.39 mg. per ml. as compared with the unfrozen juice sample.

However, storage of corn leaves or juice from the same samples of corn leaves in a cold storage room at approximately 0° C. for a period of over a month had no effect on the sucrose-reducing sugar equilibrium (unpublished data).

Procedure for analysis

STANDARDS

The proposed methods for nitrogen compounds, with the exception of nitrate, are all based on the colorimetric determination of ammonia by Nesslerization after alkaline aspiration. The sugar methods depend on the reduction of phospho-molybdate reagent by cuprous oxide and comparison

of the resulting blue solution with a standard in a colorimeter. No one set of working standards can be recommended because of the great variations in concentrations of plant constituents, depending on a number of factors. The dilute stock standards generally used were as follows: 40 mg. ammonia nitrogen per liter from ammonium sulphate, 200 mg. glucose (Bureau of Standards) per liter, and 10 mg. nitrate nitrogen per liter from potassium nitrate.

TABLE II

TOTAL AMIDE-NITROGEN IN CLARIFIED AND UNCLARIFIED BEET JUICE

TREATMENT	AMIDE-NITROGEN PER MILLILITER OF PLANT JUICE	
	10% H_2SO_4	20% $NaHSO_3$
	mg.	mg.
Unclarified	0.0167	0.0117
Clarified*	0.0020	0.0050
Unclarified	0.0696	0.0486
Clarified	0.0150	0.0147

* Clarified by 20 per cent. tannic acid solution. Preformed ammonia removed from all samples.

Ammonia is aspirated into approximately 0.01 normal sulphuric acid and determined in all cases by Nesslerization. After aeration the samples are transferred to 50-ml. volumetric flasks and made up to within 3 ml. of the mark, mixed, and Nessler's reagent added. Aliquots are so chosen that the ammonia nitrogen concentration in the final dilution is never greater than 0.0016–0.004 mg. per ml.

If a photoelectric colorimeter is available the use of transmission-concentration curves dismisses the need of working standards.

PREPARATION OF SAMPLE

Grind the sample in a Nixtamal mill or freeze with solid carbon dioxide and allow to thaw. Express the juice by means of a hydraulic press and centrifuge the resulting juice. Determine total solids by the Abbé refractometer and specific gravity with a hydrometer. Dry a fresh leaf sample in an oven at 100° C. overnight for total moisture.

TOTAL SOLUBLE NITROGEN

Filter plant juice through asbestos to remove all suspended material. Pipette a suitable aliquot and 1 ml. of an acid mixture[3] into a large Pyrex test tube and add a glass bead. To insure the reduction of nitrate add a small amount of zinc, and heat if necessary. Heat over a small flame to complete digestion. Place the tubes in an aspiration block, make the usual connections, add 1 ml. of toluene and sufficient sodium hydroxide to make the mixture alkaline, and aspirate.

For soluble inorganic nitrogen, an alternative procedure is available (11).

[3] 5 ml. 5 per cent. $CuSO_4$, 30 ml. 85 per cent. H_3PO_4, 10 ml. concentrated H_2SO_4. This combination is mixed 1:1 with water and used as suggested in the text.

Place an aliquot in the tubes in the aspiration block, add Devarda's alloy, and then alkali, causing reduction of nitrates during the alkaline rather than the acid stage. This requires a long aspiration period.

NITRATE NITROGEN (10)

Pipette a juice aliquot, 1 or 2 ml., in a 100-ml. volumetric flask containing 50 ml. water and 1 or 2 ml. of 5 per cent. $CuSO_4 \cdot 7 H_2O$, mix and add a sufficient volume of saturated Ag_2SO_4 solution to precipitate chlorides. Finally add enough solid $Ca(OH)_2$ $MgCO_3$, $4 + 10$ mixture, to precipitate excess copper. Make to volume, mix, and filter through a dry paper. Evaporate a 25-ml. aliquot of the sample to dryness. Prepare a standard by evaporating 10 ml. of a solution containing 10 p.p.m. of N from KNO_3 to dryness. Add 1 ml. of phenoldisulphonic acid solution and allow to react with the nitrate. Then add water followed by sodium hydroxide until the characteristic yellow color appears. Dilute the standard and sample to 100 ml. and compare in a colorimeter.

AMMONIA NITROGEN (24)

Add 1 to 5 ml. of juice to 2.5 ml. of permutit (FOLIN) in an ammonia aspiration tube and shake at least three minutes. Remove extraneous juice by washing with water and decanting a sufficient number of times to remove all color. To the permutit add approximately 5 ml. of water and 1 ml. of toluene. Place the tubes in an aspiration block and add 4 ml. of 10 per cent. $NaOH$; connect the apparatus and aspirate 2.5 hours into approximately $0.01 N H_2SO_4$. To reduce the time, the reaction tubes may be immersed in a water bath at 55–60° C. and aspirated 30 minutes. If ammonia is determined by Nessler's reagent the normality of the acid need only be approximate.

CLARIFICATION OF JUICE SAMPLE FOR THE DETERMINATION OF AMIDE AND ALPHA-AMINO NITROGEN

Treat 10 ml. of juice with 5 ml. of 5 per cent. sodium tungstate in 0.33 N H_2SO_4. After proteins and colored substances have been precipitated, centrifuge to remove suspended material. Pour a sample of the supernatant liquid into a test tube and shake with a sufficient amount of permutit to remove preformed ammonia.

AMIDE NITROGEN (27)

·Pipette 5 ml. or less of the protein- and ammonia-free juice into an ammonia aspiration tube; add 1 ml. 6 N H_2SO_4 and sufficient water, if necessary, to make the total volume 6 ml. At the same time place 10 ml. of phosphate buffer pH 6.0–6.5 in a second ammonia tube and add a suitable aliquot of the protein and ammonia-free juice. Place both tubes, fitted with stoppers containing capillary tubes, in a boiling water bath. Hydrolyze the first tube three hours for total amide nitrogen, and the second tube two hours for glutamine amide nitrogen. At the end of the period, cool the tubes, place in an aspiration block, and add 1 ml. of toluene. For total

amides add 3–4 ml. of a 10 per cent. NaOH solution.   To make the glutamine tube alkaline add 2 ml. of 52 per cent. K_2CO_3.   Aspirate and determine ammonium nitrogen as above.   The difference between total amide nitrogen and glutamine amide nitrogen is considered to be asparagine amide nitrogen.

AMINO NITROGEN (28)

Transfer a protein- and ammonia-free sample containing not more than 0.20 mg. alpha-amino nitrogen into an ammonia tube, add 1 ml. phosphoric acid (2 ml. 85 per cent. H_3PO_4 + 1 ml. H_2O) and 0.5 ml. ninhydrin solution (15 mg.).   Close the tube with a stopper containing a capillary glass tube 5 cm. long.   Place in a boiling water bath for 45 minutes.   Remove to an aspiration block, add 4 ml. saturated NaOH solution and aspirate 30 minutes at 50–60° C., or two hours at room temperature.   Determine ammonia by titration or Nesslerization.   In samples where glutamine and asparagine are extremely high due to abnormal environmental conditions, it is advisable to utilize the sample used for the determination of total amides.   After the removal of total amide nitrogen as ammonia, adjust the contents of the tube containing the alkaline mixture to pH 1.0 with concentrated phosphoric acid, add 15–30 mg. of ninhydrin, and place the tube in a boiling water bath for 45 minutes.   After cooling determine the ammonia by alkaline aspiration as previously described.

CLARIFICATION OF JUICE FOR THE SUGAR DETERMINATION

Pipette an aliquot of juice, 1 or 2 ml., into a centrifuge tube (15 or 50 ml.) and dilute to 10 ml.; add 1 or 2 ml. saturated neutral lead acetate solution and centrifuge.   Add 10 per cent. Na_2HPO_4 solution until the sugar solution is blue to bromthymol blue, dilute to volume, and again centrifuge.

DETERMINATION OF TOTAL SUGAR (25)

Transfer an aliquot of the clarified plant juice to a FOLIN sugar tube, add 2 drops of a 0.5 per cent. Wallenstein's invertase scale preparation. After letting stand for two hours at room temperature, make the solution alkaline and add 2 ml. of Folin alkaline copper tartrate (9).   Place the tubes in a boiling water bath for 15 minutes, remove and cool, and add the acid molybdate solution for color development; make the solution to volume.

REDUCING SUGAR

Determine this fraction in the lead-free, clarified juice, using a 1- or 2-ml. aliquot, by the Folin alkaline tartrate method mentioned above.

NON-SUGAR REDUCING SUBSTANCE (26)

Place 2 ml. of a thoroughly washed 10 per cent. yeast suspension in a 15-ml. centrifuge tube and centrifuge to destroy the suspension.   Pour off the supernatant liquid and dry the sides of the tube thoroughly with strips of filter paper.   Add an aliquot of clarified juice or fresh juice, about 4 ml., and stir.   The tube is placed in a water bath at 37–39° C. and agitated frequently.   Centrifuge after 20 minutes and determine residual reducing

power of the sample.   This value is used to correct the results for total and free reducing sugars.

Summary

This paper summarizes efforts during a considerable period to select and adapt chemical methods for the analysis of plant juice.   Most of the procedures are applicable to other types of plant extracts.   The nitrate and sugar methods, however, cannot be used with alcohol or water extracts if a visual colorimeter is used because the precipitating reagents involved do not give the necessary colorless filtrates.

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LITERATURE CITED

1. ANDRE, G. Sur la composition des liquides qui circulent dans la végétal; variations de l'azote dans les feuilles.   Comp. Rend. Acad. Sci. (Paris) **142**: 106–108. 1906.

2. Association of Official Agricultural Chemists.   Official and tentative methods of analysis. p. 622. 1935.

3. BIDWELL, G. L., and STERLING, W. F.   Preliminary notes on the direct determination of moisture. Jour. Assoc. Official Agr. Chem. **8**: 295–301. 1934.

4. BURRELL, R. C., and PHILLIPS, T. G.   The determination of nitrate nitrogen in plants. Jour. Biol. Chem. **65**: 229–334. 1925.

5. CHIBNALL, A. C., and WESTALL, R. G.   The estimation of glutamine in the presence of asparagine. Biochem. Jour. **26**: 122–132. 1932.

6. DIXON, H. H., and ATKINS, W. R. G.   Osmotic pressure in plants. Methods of extracting saps from plant organs. Sci. Proc. Roy. Dublin Soc. n.s. **13**: 422–438. 1913.

7. DONEEN, L. D.   Method for the preparation of green plant material for extraction of juice. Plant Physiol. **9**: 839–843. 1934.

8. FIFE, J. M., and FRAMPTON, V. L.   The effect of carbon dioxide upon the pH and certain nitrogen fractions of the sugar beet plant. Jour. Biol. Chem. **109**: 643–655. 1935.

9. FOLIN, OTTO.   Two revised methods for blood sugar determination. Jour. Biol. Chem. **82**: 83–93. 1929.

10. FREAR, D. E.   Estimation of nitrate nitrogen in plant juices. A study of the expression and clarification of the juice. Plant Physiol. **5**: 359–371. 1930.

11. GERDEL, R. N.   Determination of the inorganic nitrogen in the corn plant by the expressed sap method. Plant Physiol. **7**: 517–526. 1932.

12. GORTNER, R. A., LAWRENCE, J. V., and HARRIS, J. A.   The extraction of sap from plant tissue by pressure. Biochem. Bull. **5**: 139–142. 1916.

13. ——————, and HOFFMAN, W. F.   Determination of moisture content of expressed plant tissue fluids. Bot. Gaz. **74**: 308–313. 1922.

14. GREATHOUSE, G. A. The effects of the physical environment on the physico-chemical properties of plant saps and the relation of these properties to leaf temperature. Plant Physiol. 7: 349–390. 1932.

15. HARPER, J. H. The accurate determination of nitrates in soils. Ind. & Eng. Chem. 16: 180–183. 1924.

16. KENDRICK, A. B., and HANKE, M. E. The use of iodine and other modifications in the Van-Slyke manometric nitrogen method. Jour. Biol. Chem. 117: 161–174. 1937.

17. NEWTON, R., and MARTIN, W. M. Physico-chemical studies on the nature of drought resistance in crop plants. Canadian Jour. Res. 3: 336–383. 1930.

18. ORCUTT, F. S., and WILSON, P. W. Biochemical methods for the study of nitrogen metabolism in plants. Plant Physiol. 11: 713–719. 1936.

19. PUCHER, G. Reported at meeting at the Northeastern Section of the Amer. Soc. of Plant Physiol., Amherst, Mass. May 25, 1934.

20. ——————, and VICKERY, H. B. A method for determining glutamine in plant tissue. Ind. & Eng. Chem. Anal. Ed. 12: 27–29. 1940.

21. SASTRI, B. N., and SREENIVASAYA, M. The study of plant tissue fluids, a critical review. Current Sci. 3: 9–11, 52–58, 150–151. 1934.

22. SAYRE, J. D., and MORRIS, V. H. The use of expressed sap in physiologic studies of corn. Plant Physiol. 6: 139–148. 1931.

23. ——————, and ——————. Use of expressed sap in determining the composition of corn tissue. Plant Physiol. 7: 261–272. 1932.

24. SCHLENKER, F. S. Comparison of existing methods for the determination of ammonia nitrogen and their adaptability to plant juice. Plant Physiol. 7: 685–695. 1932.

25. ——————. The quantitative microanalysis of plant juice for reducing sugars and sucrose. Jour. Biol. Chem. 102: 29–34. 1933.

26. ——————. Non-reducing substances in plant juices. Jour. Biol. Chem. 117: 727–731. 1937.

27. ——————. Determination of ammonia, asparagine and glutamine in plant juice. Plant Physiol. 15: 701–709. 1940.

28. ——————. Unpublished results.

29. STUART, N. W. Determination of amino nitrogen in plant extracts. Plant Physiol. 10: 135–148. 1935.

30. VAN-SLYKE, D. D. A method for quantitative determination of aliphatic amino groups. Jour. Biol. Chem. 9: 185–204. 1911.

31. VICKERY, H. B., PUCHER, G. W., CLARK, H. E., CHIBNALL, A. C., and WESTALL, R. G. The determination of glutamine in the presence of asparagine. Biochem. Jour. 29: 2710–2720. 1935.

EFFECT OF SALT CONCENTRATION, KIND OF SALT, AND CLIMATE ON PLANT GROWTH IN SAND CULTURES[1]

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(WITH NINE FIGURES)

Introduction

Many western farmers believe that soil alkali is more detrimental to crop growth during the hot weather than during the cooler seasons of the year. Other agricultural workers have noted that damage to crops due to alkali (soil salinity) is more serious in the hot interior valleys than along the coast where the climate is more moderable. It is not definitely known whether or not soil salinity concentrations are comparable between different seasons of the year or between respective areas. Yet, information on this question is pertinent to the development of management practices which would aid in ameliorating soil salinity conditions at different seasons of the year and under different climatic conditions.

In order to obtain definite information on this question, the Regional Salinity Laboratory planned a series of experiments to determine the tolerance of crop plants to the salts commonly found in irrigation waters and soils, and to what extent climate modified these effects. This paper presents a resumé of the more pertinent results from these experiments conducted during 1939, 1940, and 1941.

TRELEASE and LIVINGSTON (10) were among the earliest authors to find that climatic conditions modified the crop growth obtained with the same culture solutions and to suggest that studies should be made on the effect of climate in modifying plant responses.

In 1938 AHI and POWERS (1) grew salt grass, alfalfa, and strawberry clover in the same culture solutions in greenhouses held at 55° and 75° F. They found germination and growth to be poorer at the higher temperature. HAYWARD and LONG (6), and WALL and HARTMAN (11) have also published results indicating that climatic factors modify the action of salt on plants.

The amount of salt which plants can tolerate in sand cultures under normal climatic conditions has been reported by many authors, and the reader is referred to recent reviews by EATON (5), and HAYWARD and LONG (6). In general, salt tolerant plants can continue growth in nutrient solution at concentrations exceeding 6 atmospheres, while salt sensitive plants may succumb at solution concentrations below 2.5 atmospheres.

Experimentation

The work was done during the summers of 1939, 1940, and 1941 in large

outdoor sand cultures at three locations, each having a distinctly different climate. These locations were Torrey Pines, near San Diego; Riverside; and Indio, in the Coachella Valley. The location of these places in Southern California is shown in figure 1. In spite of the diversity of climates the three locations were so near each other that the work could be adequately supervised and coordinated from Riverside. The stations were also in the same approximate latitude so that length of day was essentially the same at all three.

The sand culture tanks have been described by EATON (4) and each had a growing area of 3.248 square meters and a reservoir of culture solution containing 240 liters. By means of motor pumps and time clocks the sand bed could be flooded with the culture solution at definite time intervals, thus bathing the plant roots in the culture solution, providing aeration, and insuring against accumulation of salts on the surface of the sand. The culture
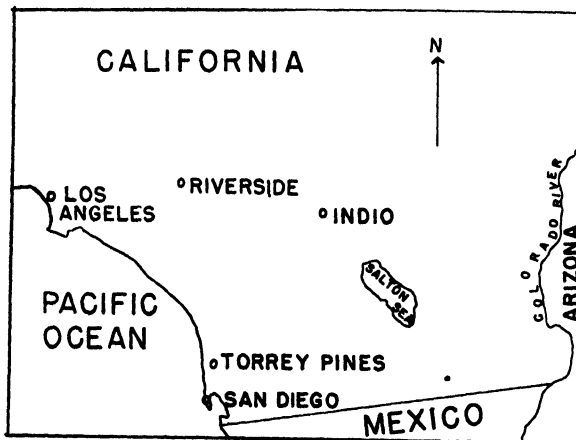

FIG. 1. Outline map of Southern California showing location of salinity experiments to test effect of climate. These tests were conducted at Riverside, Indio, and Torrey Pines.

solution drained through the sand to the reservoir below. The sand contained 0.2 per cent. magnetite to provide the iron needed by crops as suggested by CHAPMAN (2). The composition of the culture solutions used in 1941 is given in table I. They were maintained at about pH 6.5.

The salts used in making up these solutions were carefully selected for low contents of boron and heavy metals.

Growth responses obtained in these culture solutions are believed to be qualitatively characteristic of plant growth in soil solutions of equal salt content, but it should be borne in mind that drainage, aeration, nutrient supply and other growth factors will differ in soils from conditions found in sand culture beds.

EFFECT OF SALT CONCENTRATION

Early investigators, who conducted culture solution experiments to explain the action of alkali on crops in the field, have shown that at high salt concentrations growth is reduced. In figure 2, results of this nature are

TABLE I

COMPOSITION AND CHARACTERISTICS OF CULTURE SOLUTIONS USED IN 1941

TREATMENT	CATIONS PER LITER			ANIONS PER LITER		TOTAL ANIONS* PER LITER	TOTAL SALTS	CONDUCTANCE	OSMOTIC CONCENTRATION
	Na	Ca	Mg	Cl	SO$_4$				
	mg. eq.	mg. eq.	mg. eq.	mg. eq.	mg. eq.	mg. eq.	p.p.m.	$K \times 10^5$ @ 25° C.	Atmospheres
Base nutrient	2.6	3.2	2.3	2.5	1.8	10.7	1046	125	0.4
2.4 chloride	36.4	8.4	15.3	54.5	1.8	62.7	3929	649	2.4
4.4 chloride	68.9	13.2	27.8	104.5	1.8	112.7	6697	1137	4.4
2.4 sulphate	58.5	11.8	23.8	2.5	87.8	96.7	6896	753	2.4
4.4 sulphate	121.6	21.5	50.1	2.5	184.8	193.7	13520	1342	4.4
4.4 chloride sulphate mixture	87.1	16.2	34.8	67.5	67.8	141.7	9119	1195	4.4

* All solutions also contained 3.45 mg. eq. of K; 6.0 mg. eq. of NO$_3$; 0.4 mg. eq. of H$_2$PO$_4$; and boron 0.6 p.p.m.; Mn 0.2 p.p.m. These are included in the totals above.

shown for Early Wonder garden beets, Early French forcing carrots, and Weber wax beans grown in culture solutions of varying chloride concentration at Riverside during 1941.

The nutrient concentrations reported in figure 2 are given in terms of atmospheres of osmotic concentration. It is recognized that workers in the field of salinity have used a number of other indices to measure the concentration of salt on a soil or soil solution basis, such as parts per million, milligram equivalents per liter, equivalents per million, conductance[2] of a soil paste, and conductance of a soil extract, in addition to osmotic values of soil solutions or culture solutions. In this paper osmotic concentration
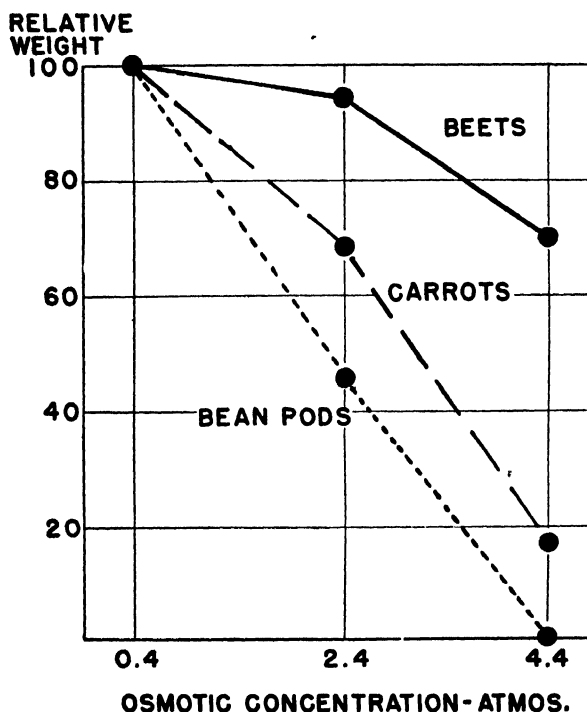
FIG. 2. Differential effect of culture solutions of varying concentration on growth of various plant species.

in atmospheres of the culture solution as determined by freezing point depression is used as our basic index. There seems to be increasing evidence (4, 5, 8) that water absorption is related to, or a part of, salinity effects, and osmotic values correlate with such phenomena. Table I gives values for the culture solutions used in 1941 in terms of milligram equivalents per liter, parts per million, conductance, and osmotic concentration.

The results shown in figure 2 are representative of a number of tolerance tests and were obtained at Riverside in the chloride series. The yield of plants grown in the base nutrient solution can be taken as 100 per cent., and

[2] Specific electrical conductances are given in reciprocal ohms and multiplied by 10[5] to avoid decimals. The values are therefore given in terms of K × 10[5] at 25° C.

yields obtained in the 2.4 and 4.4 atmosphere culture solutions plotted as a percentage of the base yield. When this is done the relationship of yield to substrate concentration is nearly linear.

It is clear from the data in figure 2 that in the case of beans the percentage decrease in growth from 0.4 atmospheres to 2.4 atmospheres is equal to the decrease from 2.4 atmospheres to 4.4 atmospheres. The yield of carrot roots shows the same uniform percentage decrease in growth for each additional atmosphere of nutrient concentration. In the case of garden beets the yield at 2.4 atmospheres is greater than the mean of yields at 0.4
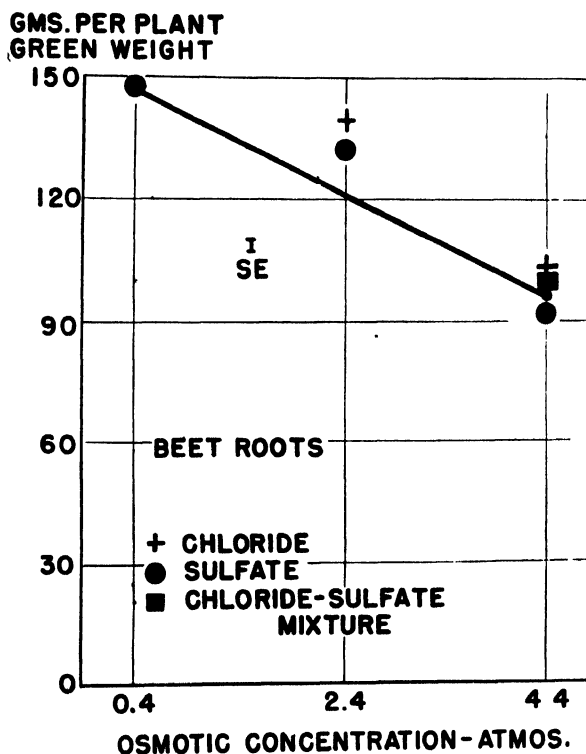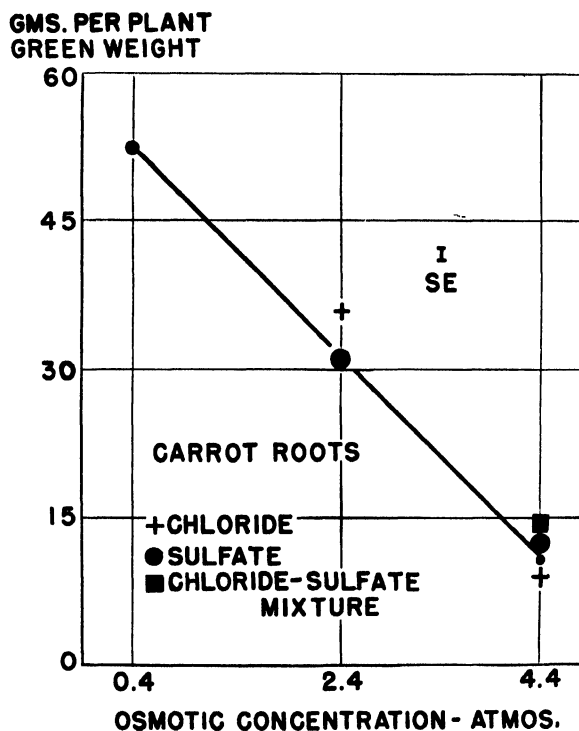

FIG. 3. The relative yield of garden beet roots grown in culture solutions of chloride, sulphate, and mixed chloride-sulphate salts at indicated osmotic concentrations. A measure of the accuracy is given by the length of the line S.E. (Standard error).

and 4.4 atmospheres, but the deviation from this mean is not statistically significant.

The relationship shown between yield and osmotic concentration is almost equally good between yield and conductance of the culture solution. This follows because conductance and osmotic concentrations of the solutions are directly related. This is shown by data in table I. These data show that the ratio of conductance to atmospheres of osmotic concentration lies between 250 and 300, depending on concentration and the nature of the salt present.

DIFFERENCES IN SALT TOLERANCE EXHIBITED BY VARIOUS PLANT SPECIES

Field experience has shown that some plants like Atriplex, Russian olive, sugar beets, and cotton have a high tolerance to salts, while others, such as beans and squash, are much less tolerant. Differences in tolerance to salinity exhibited by plant species are shown by the data in figure 2. The slope of the yield line is an index of tolerance, and beans with a steep slope are the least tolerant of the three crops shown. In fact at 4.4 atmospheres no fruit yield was obtained in the case of beans whereas a 68 per cent. yield of beets was harvested at this same concentration. Yield records at River-
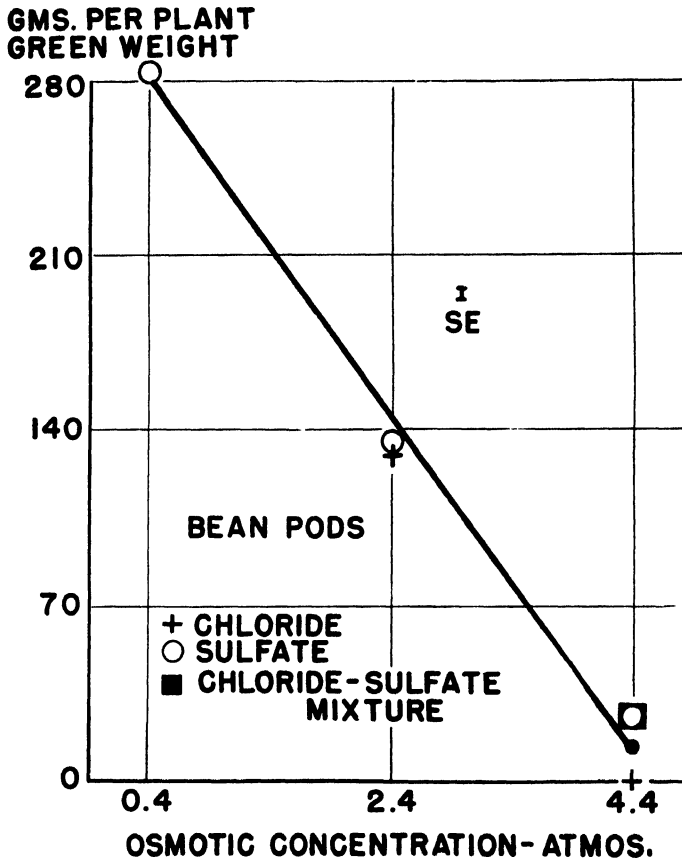

FIG. 4. The relative yield of carrots grown in culture solutions of chloride, sulphate, and mixed chloride-sulphate salts at indicated osmotic concentrations. A measure of the accuracy is given by the length of the line S.E. (Standard error).

side in the presence of chloride solutions suggest the following order for decreasing crop tolerance: Sugar beets, table beets, cotton, alfalfa, cowpeas, tomatoes, milo maize, carrots, squash, onions, and beans.

EFFECT OF ANION

The ions most prevalent in saline irrigation waters are sodium, calcium, and magnesium among the cations; and sulphates, chlorides, and bicarbonates among the anions. Almost always these six ions are found in the irrigation water or soil solutions and earlier investigators have studied the systems with a view toward determining if any one or more of the ions was

particularly toxic to plant growth.   Such experiments have not been easy to conduct because equal quantities of cations and anions must be present in a culture solution, as well as the necessary plant foods.   The action of particular anions has usually been obtained by comparison with equal moles or equivalents of anions in combination with the same cations in the culture medium.   The results of experiments in 1939 and 1940 in which equal moles of chlorides and sulphates were compared suggested that comparable re-

FIG. 5.   The relative yield of beans grown in culture solutions of chloride, sulphate, and mixed chloride-sulphate salts at indicated osmotic concentrations.   A measure of the accuracy is given by the length of the line S.E. (Standard error).

sponse might be secured if the culture solutions were prepared on an equal osmotic basis.   This was done in 1941.   The yields obtained on this basis for beets, carrots, and beans are shown in figures 3, 4, and 5.   At equal osmotic concentrations the yield of these crops did not differ appreciably.

The data obtained in 1939 and 1940 for alfalfa show that chlorides were more toxic than sulphates at approximately equal osmotic values.   This has also been found to be true for peaches (7).   These results would indicate that for some crops chlorides and sulphates at equal osmotic concentrations

are equally harmful, while with other crops, chlorides are more toxic than
sulphates at approximately equal osmotic values.   More equivalents of sul-
phaces than of chlorides are required to produce a given freezing point
depression or osmotic value.   Furthermore, the sulphate ion is somewhat
heavier than the chloride ion.   These facts explain why plants can withstand
far greater amounts of sulphate than chloride when expressed on a parts per
million basis.

EFFECT OF CATION

During 1939, sand culture tests were conducted which included treat-
ments in which calcium, magnesium, and sodium each predominated.   Thus
in one treatment 95 equivalents out of a total of 111 were supplied as cal-
cium; in another 95 out of 112 were supplied as magnesium; and in a third
47 out of 62 were supplied as sodium.   Other treatments usually had 50 per
cent. of the cations as sodium and 25 per cent. each as calcium and mag-
nesium.   Using data from 8 crops, some of them at 3 locations, and making
comparisons at approximately equal osmotic concentrations yields were
found to be slightly better when calcium was the predominant cation than
when the ratio was 50 per cent. sodium, 25 per cent. calcium and 25 per cent.
magnesium.   The increase in yield due to a predominant calcium salt was
particularly marked in the case of alfalfa.

Where magnesium was the predominant cation, yields were usually lower
than when calcium or sodium was preponderant.

Where sodium constituted 76 per cent. of the cations present in terms of
milliequivalents per liter, the yields were approximately the same as when
the ratio of sodium to total cations was 50 per cent.   Thus in sand cultures
where the physical condition of the substrate is not a problem, sodium in the
ratios used does not appear to be a particularly injurious cation for the
plants grown.

EFFECT OF CLIMATE IN MODIFYING THE EFFECT OF SALTS ON
PLANT GROWTH

It was believed that the effect of a certain salt concentration in reducing
the relative yield would not be the same in two widely different climates.
Thus if 2.4 atmospheres will reduce the yield of beans to 50 per cent. of
normal at Riverside, should we expect the same yield reduction in a hotter
climate where transpiration would be greater?   This is an important ques-
tion because if experimental data showed that climate did not modify the
effect of salts on plant growth, it would be possible to carry on experiments
at one location and expect similar results to be obtained under all climatic
conditions.

In 1940 onions were grown at the three locations in a base nutrient solu-
tion, and in this base nutrient solution plus added chloride, sulphate-chloride
mixture, and sulphate salts which had osmotic concentrations of 4.1, 4.3, and
4.5 atmospheres, respectively.   Data on climate for the month of July at
the three test locations are given in table II.

TABLE II

CLIMATIC DATA FOR INDIO, RIVERSIDE, AND TORREY PINES FOR JULY, 1940

LOCALITY	MEAN MAXIMUM TEMPERATURE	MEAN MINIMUM TEMPERATURE	AVE. EVAPORATION	AVE. RELATIVE HUMIDITY AT 8:00 A.M. & NOON		AVE. WIND	AVE. SUNSHINE
	°F.	°F.	ml./100 sq. cm./day	%	%	mi./day	gm. cal./day
Indio	112	71	1384	89	21	26	716
Riverside	97	56	1061	78	32	33	628
Torrey Pines	78	59	544	72	67	31	678

* Gram calories per sq. cm. of horizontal surface per day. The record for Torrey Pines was based on July 4 to July 21, 1940.

From data given in table II we note that sunshine at the three locations during July is nearly equal. At Torrey Pines, because of its location within 1 mile of the ocean, relative humidities are high and temperatures are equable. At Indio, on the other hand, temperatures are high, noon humidi-
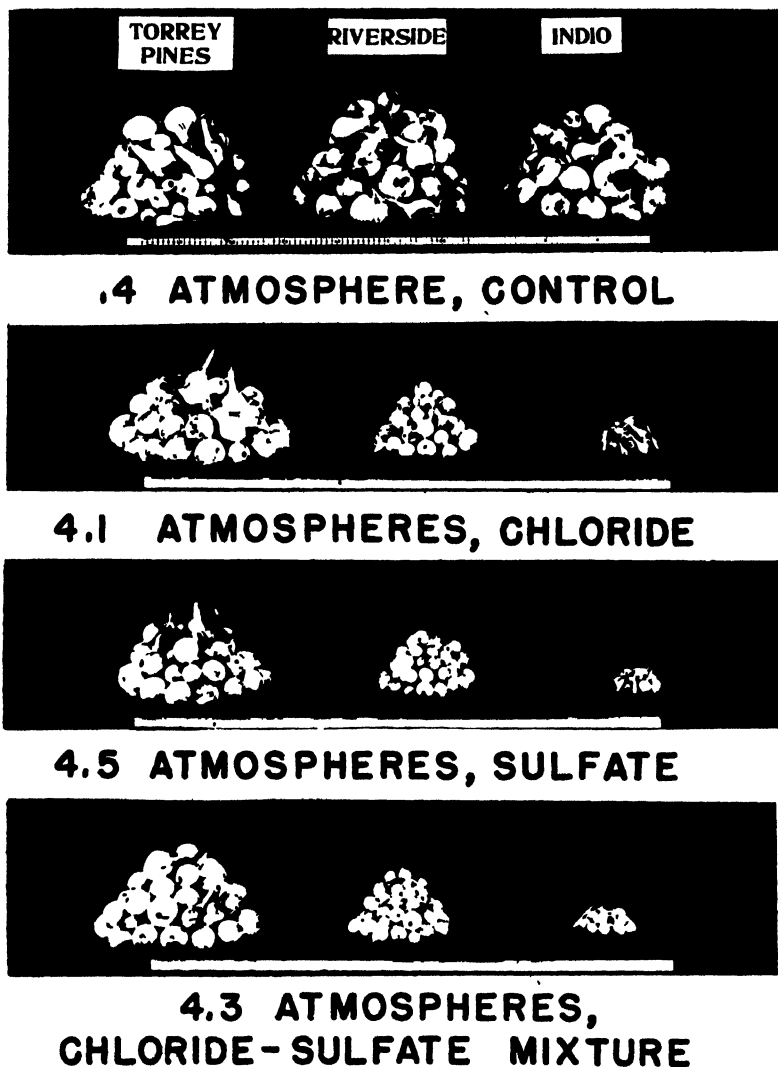

.4 ATMOSPHERE, CONTROL

4.1 ATMOSPHERES, CHLORIDE

4.5 ATMOSPHERES, SULFATE

4.3 ATMOSPHERES, CHLORIDE-SULFATE MIXTURE

FIG. 6. Harvest yields of onions grown at three locations and at varying osmotic concentrations of nitrate.

ties are low, and evaporation is high. Riverside has an intermediate climate with warm days and cool nights. Onions were grown during the period April 16 to July 26, 1940, and during the earlier part of this growth period the climatic conditions at the three stations were more similar than in July.

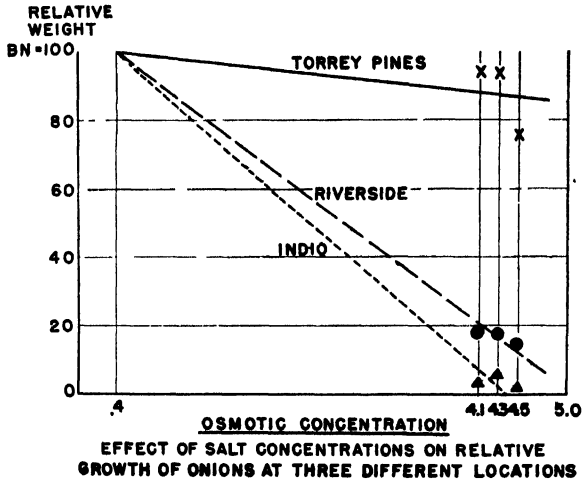A picture showing onion yields is shown in figure 6.

FIG. 7. Effect of salt concentration on relative growth of onions at three different locations.

One can compare the effect of salt, of climate, and of climate on salt effect at the three stations when the yields are placed on a common basis with
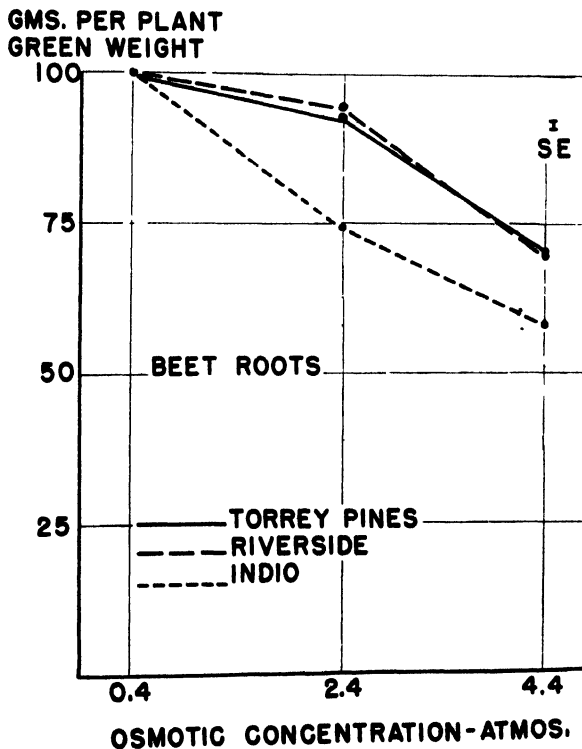

OSMOTIC CONCENTRATION-ATMOS.

FIG. 8. Effect of salt concentrations on relative growth of roots of garden beets at three different locations. A measure of the accuracy is given by the length of the line S.E. (Standard error).

yield at each location in the base nutrient taken as 100. Actual yields in the base nutrient cultures at Indio, Riverside, and Torrey Pines were 5913, 6878, and 4577 grams of fresh bulbs respectively.

The data in figure 7 have been prepared on the relative basis and show that the yield of onions was only slightly reduced at the high salt concentrations at Torrey Pines. At Indio the reduction in relative growth at osmotic concentration of 4.1 to 4.5 atmospheres was very great, nearly 100 per cent. At Riverside the relative growth reductions were nearly as great as at Indio. This test involved three replicates and the regression lines are all significantly different from each other.
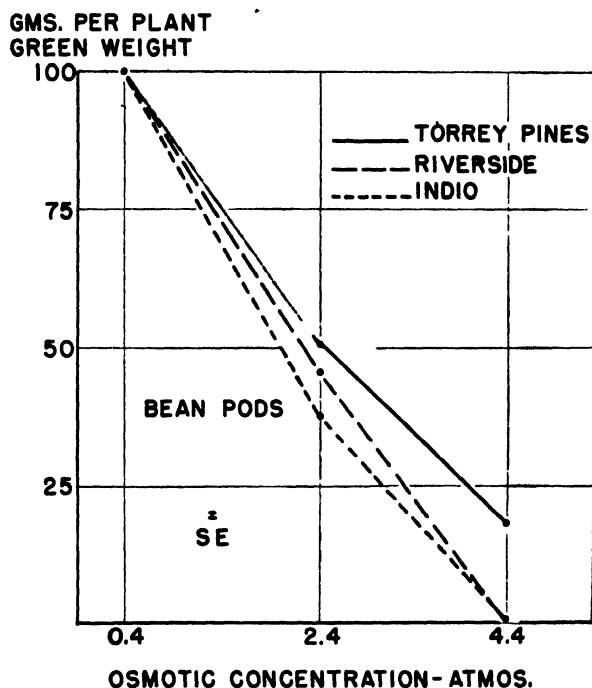

FIG. 9. Effect of salt concentrations on relative growth of beans at three different locations. A measure of the accuracy is given by the length of the line S.E. (Standard error).

When compared on this relative basis the curves in figure 7 show that in a cool climate salt concentrations in the range studied did not appreciably reduce the yield of onions. In the warmer climates as at Riverside and Indio the climate has affected the yield reduction caused by salt. In statistical terms there was a very significant interaction of climate on salt.

In 1941 additional data were obtained on beans and garden beets at the three climate locations. The data for chloride solutions are shown in figures 8 and 9.

The yields for garden beet roots shown in figure 8 indicate that on a relative basis the smallest yield reduction (base nutrient—4.4 atmospheres) occurred at Torrey Pines. On the same basis the greatest reduction in yield

with concentration was obtained at Indio. The difference in relative yield at 4.4 atmospheres at Torrey Pines and Indio was highly significant.

In the case of bean pods (fig. 9) the relative yield reduction at 4.4 atmospheres was almost the same at Riverside and Indio. The differences in relative yield between Torrey Pines and Indio were highly significant.

The data obtained in 1939 are subject to considerable variation but including only data and crops which are consistent the results for the three years can be tentatively summarized as follows:

1. A group of crops at a given salt concentration are depressed in relative yield more in warm than in cool climates. These include: in 1939, squash, and tomatoes; in 1940, onions, beans, sugar beets, alfalfa, and cotton; and in 1941, garden beets, carrots, and beans. Milo grown in 1940 grew best at Riverside and was least affected by salt at the Riverside climate.

2. Another group consists of crops for which no appreciable difference was found in the yield reduction by salt at various climates. This includes cowpeas and probably alfalfa. The data on these two crops were obtained in 1939. No alfalfa stand was obtained at Indio where daily maximum temperatures averaged 110° F.

Discussion

Best growth of a crop in nutrient solution takes place at a concentration of about 0.3 to 2.0 atmospheres, depending on the nature of the nutrient solution, volume of solution to crop, nature of crop, temperature, and other factors. It would appear that the optimum concentration should furnish an ample supply of nutrients, yet have such a low osmotic value that water absorption is not markedly reduced. In the present experiment the base nutrient solution yielded excellent crops with an osmotic concentration of 0.4 atmospheres. No tests were made with slightly greater or lower concentration. EATON (5) has indicated that small concentrations of chloride salts up to 10 milliequivalents per liter tended to improve tomato growth. The results reported in this paper do not bear on this point, but in nearly every case yields at the 2.4 atmosphere concentration were lower than in the controls. An exception occurred in the case of cotton at Torrey Pines, where the higher salt concentrations made the cotton less vegetative and the yield of bolls was greater in the salt treatments than in the controls.

The relative reduction in yield with increasing salt concentration beyond an optimum is often linear when plotted on an osmotic basis. This agrees roughly with the work of HAYWARD and LONG (6) with tomatoes. They found better growth, however, at 1.5 atmospheres than at 0.5 atmosphere, but their ratio of solution volume to growth produced was relatively low. In a base nutrient series where the osmotic concentration was achieved by using greater amounts of nutrient salts the growth was better than at comparable concentrations obtained by adding sodium chloride or sodium sulphate to a 0.5 atmosphere base nutrient solution.

EATON'S (5) data can be replotted on an osmotic concentration basis. When this is done the relationship between osmotic concentration and yields of beans, milo, seed cotton, and tomato fruits is nearly linear. Sugar beets showed a decidedly greater tolerance to chloride than to sulphate salts at equal osmotic concentrations.

AHI and POWERS (1) grew salt grass, alfalfa, and strawberry clover in various dilutions of sea water. In order to plot their yield results against osmotic values the present authors determined osmotic concentrations of Pacific Ocean water at a number of dilutions. The results obtained did not agree with a linear relationship, growth being better than expected on this basis at the high concentrations.

Work at this laboratory has dealt primarily with chloride and sulphate salts. The results to date indicate that in general, the growth reduction of crops grown in saline solutions made up of chloride and sulphate salts of calcium, magnesium, and sodium is roughly proportional to the osmotic concentration or to the conductivity of the solution. Individual crops may fall out of line somewhat, but these deviations should be checked further. Specific ion effects undoubtedly exist, but for the salts studied they appear to be of a second order, compared to the matter of total salt concentration.

While the results obtained in sand cultures indicate that there is little difference in cation effects, these results are wholly tentative. It is believed that if the range of each cation is varied widely, marked plant responses will occur. Sodium has a very profound effect on soil structure and sodium saturated soil takes on physical and chemical characteristics which are not conducive to plant growth. For this reason results with sodium in sand cultures are not expected to carry over completely into soils. Just how osmotic values affect growth is not known, but undoubtedly water intake, turgidity, and root extension are involved, together with other salt effects on the root and within the plant.

It is of interest to note that the soil solution of productive soils may have an osmotic concentration exceeding 1 atmosphere at soil moisture contents within the wilting range. Soils marginal because of salinity may have osmotic concentrations as great as 10 atmospheres at these moisture contents (9). These soil solution concentrations are of the same order as the concentrations of the culture solutions used in these sand culture experiments.

The results obtained in this study show that most crops are injured by salt to a greater extent in warm than cool climates. The alfalfa data for 1939 gave no appreciable differences in the salt effect at Riverside and Torrey Pines. In 1940 when the alfalfa was seeded earlier in the season and good stands were obtained, decidedly poorer yields at the same salt treatments were obtained at Indio compared with Riverside and Torrey Pines. As in 1939, there was no appreciable difference in yields at Riverside and Torrey Pines in the comparable treatments.

The data of AHI and POWERS when plotted on a relative basis give

slightly, but probably not significantly, lower yields of alfalfa at warm temperatures in the presence of salt. Their cold temperature of 55° F. was about 10° less than the average temperature at Torrey Pines. These results would indicate that the temperature coefficient for salt injury in the case of alfalfa is low.

Because of the great differences in crop reaction to the combined effect of salt and temperature as shown in this paper it appears dangerous to extrapolate results from one set of climatic conditions to another.

Summary

1. Milo, cotton, alfalfa, sugar beets, barley, tomatoes, squash, cowpeas, onions, navy beans, garden beets, and carrots, were grown in large sand cultures in three diverse locations at various salt concentrations ranging from 0.4 to 4.5 atmospheres.

2. Total salt concentration expressed in atmospheres was a greater factor in determining the amount of growth reduction than effects caused by specific ions.

3. Growth reduction was in most cases linear with increasing osmotic concentration of substrate.

4. Conductance of the nutrient solution characterized the total salt effect probably as well as osmotic concentration.

5. Chloride and sulphate salts when compared on an equal osmotic basis, depressed growth to an equal extent with a number of crops. In the case of other crops, chloride salts were slightly more toxic than the sulphate at equal osmotic concentrations.

6. Within the ratios of cations used in the experiments, there was no great difference in the action of individual cations on plant response. Sodium did not appear to be an unduly toxic cation in sand cultures.

7. Crops do not behave alike in their reaction to the combined effect of salt and climate. Thus, some are reduced equally in relative yield at a given salt concentration irrespective of climate, while most crops at the same salt concentration are depressed in relative yield more in warm than in cool climates.

8. A number of crop species died in a culture solution having an osmotic concentration of 4.5 atmospheres.

LITERATURE CITED

1. AHI, S. M., and POWERS, W. L.   Salt tolerance of plants at various temperatures. Plant Physiol. **13**: 767–789. 1938.

2. Chapman, H. D. Absorption of iron from finely ground magnetite by citrus seedlings. Soil Sci. **48**: 309–315. 1939.

3. Eaton, Frank M. Plant culture equipment. Plant Physiol. **16**: 385–392. 1941.

4. ——————. Water uptake and plant growth as influenced by inequalities in the concentration of the substrate. Plant Physiol. **16**: 545–564. 1941.

5. ——————. Toxicity and accumulation of chloride and sulfate salts in plants. Jour. Agr. Res. **64**: 357–399. 1942.

6. Hayward, H. E., and Long, E. M. Anatomical and physiological responses of the tomato to varying concentrations of sodium chloride, sodium sulfate, and nutrient solutions. Bot. Gaz. **102**: 437–462. 1941.

7. ——————, and ——————. Vegetative responses of the Elberta peach on Lovell and Shalil rootstocks to high chloride and sulfate solutions. Proc. Amer. Soc. Hort. Sci. **41**: 149–155. 1942.

8. Long, E. M. Influence of solute concentration or intake of water and nutrients by roots of approach-grafted tomato plants. Paper presented before Bot. Soc. Amer. at Dallas, December, 1941; abstract in Amer. Jour. Bot. **28**: 12s. 1941.

9. Magistad, O. C., and Reitemeier, R. F. Composition and characteristics of soil solutions from normal and saline soils at low soil moisture contents. (Unpublished.)

10. Trelease, Sam F., and Livingston, B. E. The relation of climatic conditions to the salt proportion requirements of plants in solution cultures. Science n.s. **59**: 168–172. 1924.

11. Wall, Robert F., and Hartman, E. L. Sand culture studies of the effects of various concentrations of added salts upon the composition of tomato plants. Proc. Amer. Soc. Hort. Sci. **40**: 460–466. 1942.

AMOUNT, DISTRIBUTION, AND SEASONAL TREND OF CERTAIN ORGANIC RESERVES IN THE ROOT SYSTEM OF FIELD BINDWEED, *CONVOLVULUS ARVENSIS* L.[1]

JOHN C. FRAZIER

(WITH SIX FIGURES)

Introduction

Field bindweed, *Convolvulus arvensis,* is recognized as one of the more serious of the noxious weeds in the central United States. Four of the five federal projects established to study control measures for this plant are located in this area (Hays, Kansas; York, Nebraska; Cherokee, Iowa; and Lamberton, Minnesota).

During the past two or three decades practical means of control have been studied at various places. Empirical methods, however, have not resulted in satisfactory control and there has been a trend to include in a general research program studies dealing specifically with the physiology of the plant, especially studies of its food reserves. The persistent nature of noxious perennial weeds is intimately related to the organic reserves stored in their roots. The study herein reported deals particularly with the organic reserves of the root system when undisturbed by cultivation.

A study of the nature of the root system (10) showed the advisability of dividing it into a number of portions for detailed studies of their organic reserves. Analytical determinations were made upon 7 of these portions found in the upper 3 feet of soil, to learn the amount and relative distribution of certain of the organic reserves during the growing season.

ENVIRONMENTAL FACTORS

The environmental factors of soil type, precipitation, and air temperatures under which this study was made are given in detail by FRAZIER (10).

Briefly, it may be stated that the soil was a deep Geary silt loam, no layer of which impeded root development. The rainfall the preceding year was 24.54 inches which was 74 per cent. of normal; while during the first 10 months of 1937 the precipitation was 20.88 inches, which was 69 per cent. of normal for this period. The air temperatures the preceding year showed great extremes. On 60 days, temperatures of 100° F. or higher were reached (the average number is 15). The summer of 1937 was less extreme with temperatures of 100° being attained on only 38 days. The growth of bindweed was definitely terminated on November 17 on which date the temperature fell to 12° F.

Materials and methods

PHYSICAL METHODS

The roots used in this study were obtained by a modification of the trench-

ing method developed by WEAVER (25).   This involved the use of a hand pick and an ice pick to expose and free the underground parts after suitable trenches had been made by excavating operations.

Roots secured for analytical studies were taken twice a month—the first and fifteenth—from April 1, 1937, to October 1, 1937, with a fourteenth and final sampling on November 1, 1937.   All roots were taken from the one area of well-established, undisturbed bindweed, which was subjected to normal root competition by a uniform, but sparse stand of other weeds. There was scarcely any competition for light as the bindweed shoots twined over the other weeds.

The material was divided into the 7 portions of the root system as collected and kept in moist cloths until it was taken to the laboratory.   There it was washed free of soil particles, blotted dry with cloths, cut in pieces approximately one-fourth of an inch in length, and placed in individual wire trays.

The root material was killed in an electric oven at a temperature of 95° C.   This ordinarily required from 20 to 30 minutes.   It was then dried at 70° C. in an electric oven for at least 14 hours.

The material was ground in a food grinder and then reduced to 60-mesh size with a mortar and pestle.   It was stored in sealed glass containers, then redried at 100° C. for 14 hours before samples were taken for analysis.

One hundred lineal feet of each of the 3 portions into which the vertical roots were divided and one hundred lineal feet of the lateral roots of comparable plants growing in the same area of land were excavated and weighed in late July, 1939.

CHEMICAL METHODS

The following fractions were determined in each of the 7 divisions into which the root system was divided: total nitrogen, protein nitrogen, nonprotein nitrogen, reducing sugars, total sugars, the starch-dextrin fraction, and the readily available carbohydrate fraction.   All results are the averages of closely-agreeing duplicate analyses.

TOTAL NITROGEN.—The diphenylamine test (13) showed the material to be free of nitrates which agrees with the findings of BAKKE et al. (3) and the Kjeldahl-Gunning-Arnold method (2) as modified by MILLER (14) was used to determine the total nitrogen in 1-gram samples.

PROTEIN NITROGEN.—This fraction as determined is considered to include the unextracted nitrogen plus that coagulated by specific treatment and is the method that has been employed in this laboratory (14).

NONPROTEIN NITROGEN.—This was obtained by subtracting the percentage of the protein nitrogen from the percentage of total nitrogen.

The reducing sugars, total sugars, and the starch-dextrin fraction were determined from a 3-gram sample, using methods adapted from those employed in this laboratory (14).   The sample was extracted with 100 ml. of 80 per cent. alcohol for 2 hours in a 70° C. bath, filtered, the filtrate used for sugar, and the residue for starch-dextrin determinations.

REDUCING AND TOTAL SUGARS.—The filtrate was freed of alcohol by evaporation and the resulting water solution was cleared by the use of neutral lead acetate, brought to volume, deleaded with sodium oxalate, the lead oxalate precipitate allowed to settle, and a 50-ml. aliquot pipetted off for reducing sugar determinations. One hundred milliliters of the remaining aqueous solution were pipetted into a 250-ml. volumetric flask, acidified with 2.5 per cent. hydrochloric acid and hydrolyzed for 5 minutes at 70° C., as described by LOOMIS and SHULL (13), nearly neutralized with 20 per cent. sodium hydroxide, made to volume and a 50-ml. aliquot taken for total sugar determinations.

STARCH-DEXTRIN FRACTION.—The residue of the original separation was transferred to a beaker by means of hot water, made to approximately 100 ml. volume and boiled for 2 minutes to gelatinize the starch. After cooling, 10 ml. of fresh filtered saliva and 1 ml. of toluene were added and the contents of the beaker stirred thoroughly.

The beaker, covered with a watch glass, was placed in an electric oven at 38–40° C. for 14 hours, checked as being starch-free with iodine, filtered into a volumetric flask, washed with hot water, cooled, cleared with neutral lead acetate, made to volume, deleaded with sodium oxalate, the lead oxalate allowed to settle, and a 200-ml. aliquot pipetted into an Erlenmeyer flask, acidified with 2.5 per cent. hydrochloric acid, refluxed for 3 hours, nearly neutralized with 40 per cent. sodium hydroxide, brought to volume in a 250-ml. volumetric flask, and a 50-ml. aliquot taken for the starch-dextrin fraction determination.

All carbohydrate fractions were determined gravimetrically by weighing the cuprous oxide directly. The conditions of MUNSON and WALKER (15) were followed for reduction. The d-glucose equivalent was obtained from the MUNSON and WALKER tables.

READILY AVAILABLE CARBOHYDRATE FRACTION.—This composite fraction was computed by adding the percentage of total sugars to the percentage of the starch-dextrin fraction.

All results were obtained in percentage and in grams per 100 lineal feet for each of the reserve food fractions in each of the 7 portions into which the plants were arbitrarily divided. The weight in grams per 100 lineal feet for any reserve food fraction was obtained by multiplying the weight of 100 lineal feet of the plant portion by the percentage of the reserve food fraction in that portion.

Experimental studies

NATURE OF THE PLANT

FRAZIER (10) has described in some detail the gross nature of the undisturbed bindweed plant grown from seed. In summary, it was found that the undisturbed root system may be divided into three natural portions: (1) primary vertical root, which is the original root of the plant developing from the radicle of the seed; (2) permanent lateral roots, those lateral roots

that develop more extensively and persist as permanent parts of the root system; and (3) secondary vertical roots which are the downward extensions from the permanent lateral roots after the latter have made 10 to 30 inches of horizontal growth.

In this study all permanent lateral roots of whatever order (10) are considered together, and likewise all secondary vertical roots are considered collectively, regardless of their order of development. The two types of vertical roots were each divided into the first-, second-, and third-foot divisions or portions. These 6 divisions and the lateral-root division constitute the 7 portions of the plant into which it was arbitrarily divided for the purpose of determining the distribution and trend of organic reserves during the growing season, April 1 through November 1. The so-termed ''first foot'' of the secondary vertical roots was considered to begin where the slender lateral root suddenly enlarged markedly. This ''first foot'' was the first 12 inches of root beyond that point. Ordinarily this portion was entirely contained in the first foot of the soil. All the material designated as lateral roots came from the first-foot soil level.

Organic reserves of the plant

PERCENTAGE BASIS.—These results are reported as the percentage of the reserve food fraction in the dry material of the root portion. It was not possible to report them as percentages of the total dry weight of the entire plant since under field conditions complete plants could not be secured in sufficient numbers.

It is recognized that percentages may not give a true picture of the changes that are occurring in the actual amounts of organic reserves in the plant. An increase in the actual amount of one fraction may bring about an ''apparent'' decrease in another fraction, when reported on a percentage basis, despite the fact that there has been no actual decrease in the latter fraction. Admittedly the changes from protein nitrogen to the nonprotein nitrogen fraction and vice versa, and from sugars to the starch-dextrin fraction and vice versa, will show apparent errors in these fractions. It is contended, however, that the total nitrogen and the total readily available carbohydrate fractions are fairly reliable indices, as such shifts would be occurring within these fractions and would not materially change their values.

NITROGEN FRACTIONS.—*The primary vertical roots.*—In figure 1[2] the data are given for the total nitrogen, protein nitrogen, and nonprotein nitrogen fractions, expressed in percentage on a dry weight basis, for the 3 portions of the primary vertical roots. There is a definite decrease from April 15 to June 15, the decrease being more marked in some portions. There is less protein nitrogen relative to nonprotein nitrogen in the second, and still less in the third foot portion. The total-nitrogen and protein-nitrogen fractions reached their seasonal low points on August 15 in the second and third foot levels.

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[2] The data, other than tables I and II, on which the graphs of this paper are based, are on file at the Kansas Agricultural Experiment Station, available upon request.

The secondary vertical roots.—In figure 2 the data are given for the total nitrogen, protein nitrogen and nonprotein nitrogen, expressed in percentage on a dry-weight basis for the secondary vertical roots. There was the same early season decrease following April 15, as in the primary vertical roots. The low point in the total nitrogen and protein nitrogen fractions of these
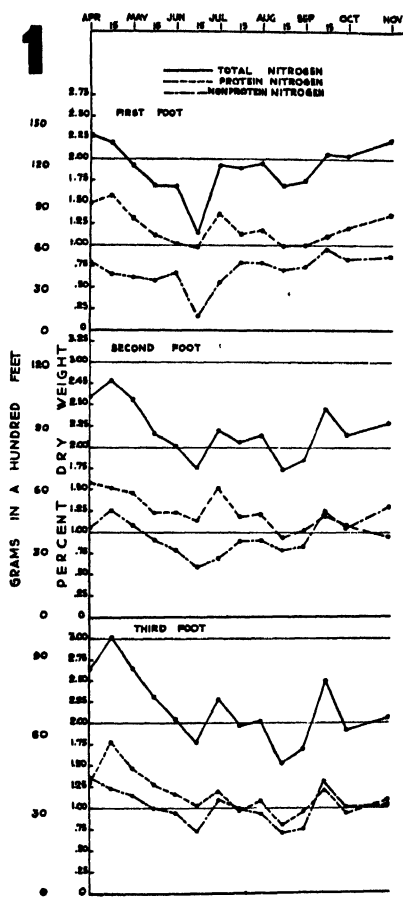

FIG. 1. The total nitrogen, protein nitrogen, and nonprotein nitrogen fractions in percentage on a dry weight basis and in grams per 100 lineal feet in the first, second, and third foot portions of the primary vertical root.
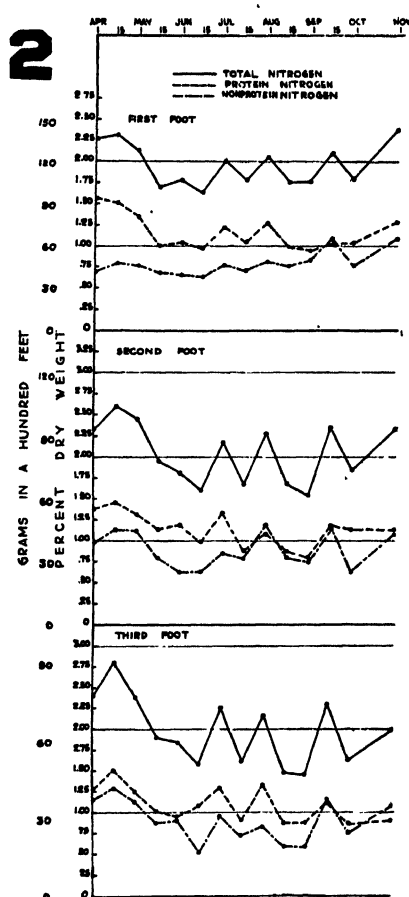
FIG. 2. The total nitrogen, protein nitrogen, and nonprotein nitrogen fractions in percentage on a dry weight basis and in grams per 100 lineal feet in the first, second, and third foot portions of the secondary vertical root.

roots centered, however, about September 1, except for the former in the surface foot. The trends after June 15 show more marked variations than those of the same fraction in the corresponding portion of the primary vertical root, but the general range of percentages does not differ markedly.

The lateral roots.—Figure 3 gives the data for the 3 nitrogen fractions in percentage of dry weight for the lateral roots. The early season decline is quite marked, especially in the total nitrogen fraction, due to the increase
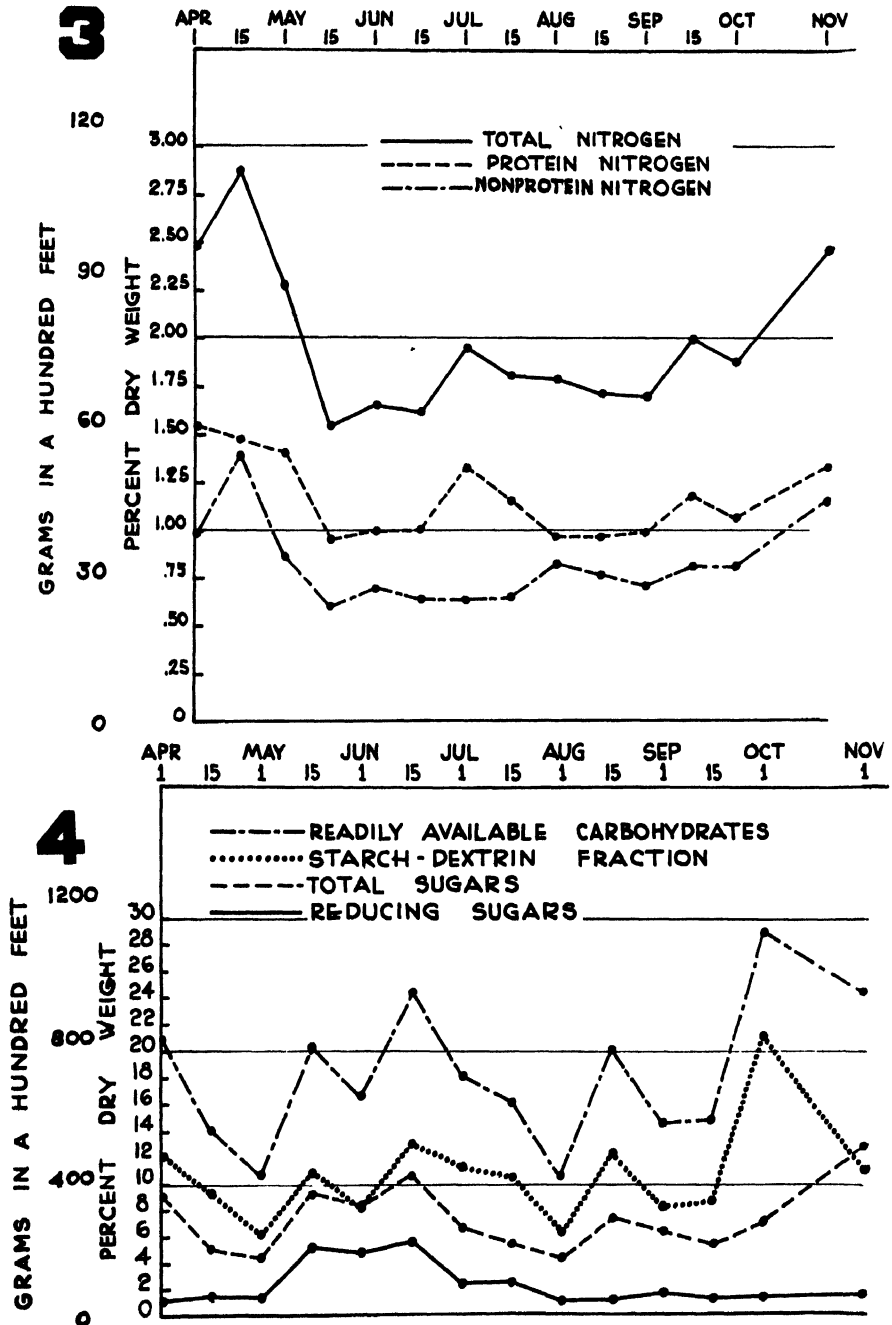
FIG. 3. The total nitrogen, protein nitrogen, and nonprotein nitrogen fractions in percentage on a dry weight basis and in grams per 100 lineal feet of the lateral root.

FIG. 4. The reducing sugars, total sugars, starch-dextrin fraction, and readily available carbohydrates in percentage on a dry weight basis and in grams per 100 lineal feet of the lateral root.

between April 1 and 15.   Lows are found in the total-nitrogen and protein-nitrogen fractions in the months of May 15 to June 15 and August 1 to September 1.   The general range of percentages of the 3 fractions is much the same as in the two types of vertical roots if the extremely low point of total proteins on June 15 in the primary vertical roots and the high point of this fraction on April 15 in the lateral roots are not allowed to bias the picture.

CARBOHYDRATE FRACTIONS.—*The primary vertical roots.*—Data are given in tables I and II for total sugars and the starch-dextrin fraction and in figure 5 for reducing sugars, total sugars, the starch-dextrin fraction, and the readily available carbohydrate fraction for the primary vertical roots.

The reducing sugars differed somewhat in their seasonal trend in the 3 portions.   The early season low was from April 15 to May 15 in the first, from April 15 to May 1 in the second, and on April 1 in the third foot. There was a decrease in the first foot portion after July 15.

The total sugars showed an early season decrease in all portions.   The seasonal low was reached by this fraction on May 15 in the first, on July 1 in the second, and on April 15 in the third foot portion.   High levels were attained on June 15 and November 1 in the first, on June 1, September 15, and October 1 in the second, and on June 15 and August 15 in the third foot portion.

The starch-dextrin fraction reached a low point on May 1 in all three foot portions.   This reserve fraction in the first foot portion was less than the total sugars on May 1 and June 15.   The high points for the starch-dextrin fraction was on October 1 for the first, August 15 and October 1 for the second, and September 1 for the third foot portion.   This fraction attained progressively higher percentages in the second and third foot portions.

The readily available carbohydrate fraction had the same general trend as the starch-dextrin fraction except between May 1 and July 1 in the first foot portion.   It also attained progressively higher percentages in the second and third foot portions.

The secondary vertical roots.—Data are given in tables I and II for total sugars and the starch-dextrin fraction and in figure 6 for reducing sugars, total sugars, the starch-dextrin fraction, and the readily available carbohydrate fraction for the secondary vertical roots.

The reducing sugars differed in their trends from those in the corresponding portions of the primary vertical root.   This fraction ran slightly higher in these roots than in comparable portions of the primary vertical root.

The total sugars reached low points on April 15, June 1, August 1, and October 1 in the first, on May 15 and October 1 in the second, and on May 15 in the third foot portion.   High levels were attained on May 1, June 15, the month of August 15 to September 15, and November 1 in the first foot (the peak on November 1), on September 1 in the second, and on June 15 in the third foot levels.   If the extremely low point reached in the first foot of the

TABLE I

TOTAL SUGARS IN PERCENTAGE AND IN GRAMS PER HUNDRED LINEAL FEET. MANHATTAN, KANSAS, 1937

| DATE | PRIMARY VERTICAL ROOT | | | | | | SECONDARY VERTICAL ROOT | | | | | | LATERAL ROOT | |
| | 1ST FOOT | | 2D FOOT | | 3D FOOT | | 1ST FOOT | | 2D FOOT | | 3D FOOT | | | |
	%	gm.	%	gm.	%	gm.	%	gm.	%	gm.	%	gm.	%	gm.
April 1	6.04	374	8.86	360	8.86	286	8.08	500	8.72	354	9.92	320	9.12	346
April 15	4.75	294	6.42	261	5.17	167	5.75	356	7.83	318	8.46	273	5.00	190
May 1	5.33	330	5.37	218	7.17	232	9.12	503	6.45	262	11.25	363	4.29	163
May 15	1.96	121	5.83	237	8.12	262	7.38	457	6.01	244	5.21	168	9.23	350
June 1	6.62	410	9.54	387	9.92	320	5.33	330	8.21	333	12.66	409	8.23	312
June 15	8.42	521	7.25	294	11.50	371	8.58	531	8.21	333	13.20	426	10.96	415
July 1	3.31	205	4.29	174	10.00	323	6.20	384	8.71	354	9.54	308	6.98	265
July 15	6.96	431	8.42	342	9.79	316	6.79	420	10.76	437	11.33	366	5.96	226
August 1	7.08	438	7.38	300	10.08	326	5.04	312	8.33	338	8.80	284	4.04	153
August 15	6.79	420	9.13	371	12.63	408	8.88	550	9.13	371	9.08	293	7.78	295
Sept. 1	7.29	451	8.29	337	9.29	300	8.45	523	12.04	489	11.58	374	6.41	243
Sept. 15	7.21	446	9.46	384	7.54	244	8.88	550	9.13	371	9.59	310	5.71	216
Oct. 1	5.06	313	9.87	401	8.50	275	5.46	338	5.69	231	9.17	296	7.54	286
Nov. 1	9.54	591	9.17	372	8.13	263	9.58	593	9.91	402	10.50	339	13.21	501

TABLE II

STARCH-DEXTRIN FRACTION IN PERCENTAGE AND IN GRAMS PER HUNDRED LINEAL FEET. MANHATTAN, KANSAS, 1937

DATE	PRIMARY VERTICAL ROOT						SECONDARY VERTICAL ROOT						LATERAL ROOT	
	1ST FOOT		2D FOOT		3D FOOT		1ST FOOT		2D FOOT		3D FOOT			
	%	gm.	%	gm.	%	gm.	%	gm.	%	gm.	%	gm.	%	gm.
April 1	5.45	337	10.85	441	23.99	775	11.05	684	20.11	816	25.28	817	12.17	461
April 15	4.98	308	12.58	511	14.29	462	7.21	446	13.96	567	17.63	569	9.05	343
May 1	1.75	108	7.81	317	11.42	369	5.60	347	11.04	448	13.08	422	6.50	246
May 15	4.50	279	9.94	404	13.79	445	8.20	508	15.10	613	18.56	599	11.38	431
June 1	7.84	485	14.12	573	18.35	593	9.33	578	16.42	667	22.52	727	8.16	309
June 15	6.02	373	9.11	370	14.85	480	15.91	985	17.73	720	26.88	868	13.75	521
July 1	6.88	426	13.28	539	17.04	550	5.28	327	15.42	626	11.66	377	11.79	447
July 15	11.44	708	18.83	765	21.60	698	12.06	747	22.79	925	26.19	846	10.75	407
August 1	11.58	717	21.39	868	26.12	844	8.04	498	19.37	786	22.17	716	6.51	247
August 15	12.10	749	22.62	918	27.58	891	14.06	870	30.77	1249	38.27	1236	12.69	481
Sept. 1	7.92	490	19.83	805	31.95	1032	14.06	870	19.25	782	33.52	1083	8.05	305
Sept. 15	8.98	556	17.50	711	22.36	722	13.85	857	19.48	791	26.61	860	8.92	338
Oct. 1	15.15	938	22.50	914	27.56	890	21.42	1326	30.68	1246	34.93	1128	21.56	817
Nov. 1	8.92	552	20.23	821	25.96	839	12.37	766	24.55	997	31.10	1005	11.08	420

primary vertical root is overlooked, however, the range of percentages is similar in the two types of vertical roots.

The starch-dextrin fraction trends were downward in all portions until May 1. In the first foot portion, the starch-dextrin fraction was less than the total sugar fraction on May 1 and July 1. The general trends were similar to those of this fraction in corresponding portions of the primary vertical root, except that marked low points were reached in the first and third foot portions on July 1. The fraction, however, ran 4 to 6 per cent. higher
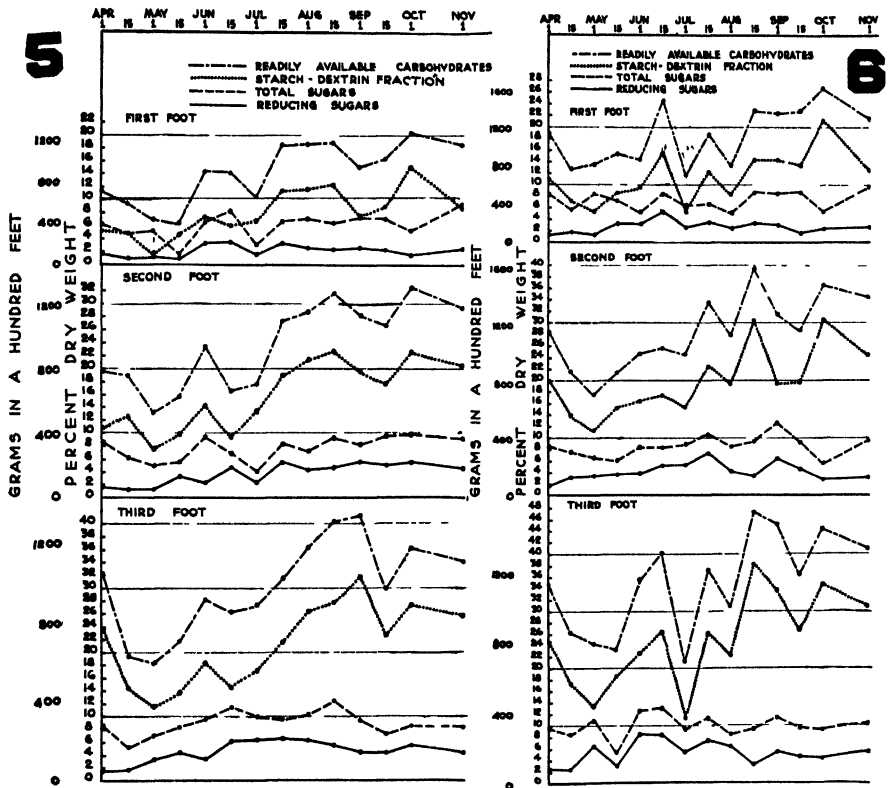
FIG. 5. The reducing sugars, total sugars, starch-dextrin fraction, and readily available carbohydrates in percentage on a dry weight basis and in grams per 100 lineal feet in the first, second, and third foot portions of the primary vertical root.

FIG. 6. The reducing sugars, total sugars, starch-dextrin fraction, and readily available carbohydrates in percentage on a dry weight basis and in grams per 100 lineal feet in the first, second, and third foot portions of the secondary vertical root.

in many instances than in the corresponding portion of the primary root.

The readily available carbohydrate fraction showed the same general trends as the starch-dextrin fraction in this root. This fraction ran 6 to 8 per cent. higher in many instances than corresponding portions of the primary vertical root.

The lateral roots.—Data are given in tables I and II for total sugars and the starch-dextrin fraction, and in figure 4 for reducing sugars, total

sugars, the starch-dextrin fraction, and the readily available carbohydrate fraction for the lateral roots.

The low point of reducing sugars occurred at the start of the study on April 1 and a second low interval existed from August 1 to August 15. The peak was attained on June 15. The general trend was similar as for this fraction in the first foot portion of the two types of vertical roots.

The total sugars reached low points on May 1 and on August 1 and attained high levels on June 15 and November 1. The general trend differed somewhat from those of this fraction in the first foot level of the vertical roots.

The starch-dextrin fraction dropped to low points on May 1 and on August 1. High points were reached on June 15 and August 15, but the peak was reached on October 1. On June 1 and November 1 this fraction was slightly less than the total sugar fraction.

The trend of the total readily available carbohydrate fraction practically paralleled that of the starch-dextrin fraction for this root. A high point was reached on June 15, with the seasonal peak being attained on October 1. The percentages of this fraction, as in the starch-dextrin fraction, more closely approximated those in the first foot of the secondary than in the first foot of the primary vertical roots.

BASIS OF ACTUAL AMOUNT.—The story of any reserve food fraction expressed on a percentage basis is confusing in that the percentage ordinarily increases with depth while the actual amount ordinarily decreases. The findings of BAKKE et al. (3) show these trends.

In order to give the results of this study in terms of the actual amounts of each of the individual reserve food fractions in each of the 7 portions into which the plant was arbitrarily divided, the weight of 100 lineal feet of each of the 3 portions into which the vertical roots were divided and 100 lineal feet of the lateral roots was obtained by excavating representative roots of comparable plants and weighing them. These weights were as follows:

Weight of 100 lineal feet

Vertical root
    1st foot portion .... 61.9 gm.
    2d ''.   ''   .... 40.6 "
    3d "   "   .... 32.3 "
    Lateral root .... 37.9 "

The weight of the individual root portion was then multiplied by the percentage of the reserve food fraction in that portion to give the actual weight of that reserve fraction in 100 feet of that portion. These data have been computed and they show that the picture of reserves from the standpoint of actual amounts is markedly different from that constructed on a percentage basis. A specific case involving the first and third foot portions of the primary vertical root for August 1 illustrates this point. On a per-

centage basis, the first foot had 18.66 and the third foot 36.20 per cent. of the total readily available carbohydrate fraction, while on the basis of the actual amount of this reserve fraction, the first foot had 1,155 grams as compared with 1,169 grams in the third foot. Hence, with almost twice the percentage of this reserve fraction, the third foot had but little more of the fraction.

Nitrogen fractions.—These computed data are given in graphic form. Figures 1, 2, and 3, inclusive, give total nitrogen, protein nitrogen, and non-protein nitrogen fractions in the primary vertical roots, the secondary vertical roots, and the lateral roots in grams per 100 lineal feet.

Carbohydrate fractions.—The computed data for total sugars and the starch-dextrin fraction are given in both tabular (tables I and II) and graphic form (figs. 4, 5, and 6), and in graphic form only for reducing sugars and the readily available carbohydrate fraction in the lateral, the primary vertical, and the secondary vertical roots, respectively.

It is obvious because of the method of calculation that the trend of the data reporting the actual amount of a reserve food fraction in grams per 100 lineal feet of a root portion will be the same as that of those reporting the percentage of this reserve food fraction in the same root portion.

Statistical treatment of differences between comparable portions of vertical roots

The seasonal trend of the various carbohydrate and nitrogen fractions was essentially the same for the primary and secondary vertical roots, hence comparable foot portions of these roots were paired by sampling dates and the differences of these various fractions in the comparable portions treated statistically, using the t test, to ascertain if the differences were statistically significant and, if so, to what degree (17).   The value of t was used to determine the probability (P) from the tables for P values given by Fisher and Yates (9).   These values are given in table III.

These P values showed that the differences observed between comparable portions of the two types of vertical roots were statistically significant in many instances.   The differences in the total nitrogen fraction in the second and third foot portions, the protein nitrogen in the second foot portion, and the nonprotein fraction in the first foot portion were at least significant. The reducing and total sugars were at least significantly different in the first foot portion, while the starch-dextrin and readily available carbo-hydrate portions were at least significantly greater in the secondary vertical roots in all three foot levels.

It would appear that the statistical significance of the differences warrants consideration. Under ordinary conditions of growth, however, the secondary vertical roots outnumber the primary ones from two to many times, which may mean that root samples taken from undisturbed bindweed are predominantly of the one type.   Then, too, the effect of control procedures, such as cultivation, on these differences is not known.

TABLE III

THE t VALUE, PROBABILITY, AND DEGREES OF SIGNIFICANCE OF THE DIFFERENCES BETWEEN
THE VARIOUS ORGANIC RESERVE FRACTIONS FOUND IN COMPARABLE FOOT PORTIONS
OF THE TWO TYPES OF VERTICAL ROOTS PAIRED BY SAMPLING DATES

FOOT INTERVAL	AVERAGE OF RESERVE FRACTION FROM 14 DATES OF SAMPLING		t VALUE (13 df.)	P (PROBABILITY)	SIGNIFICANCE
	VERTICAL ROOT				
	PRIMARY	SECONDARY			
	NITROGEN FRACTIONS				
	Total nitrogen				
	%	%			
1	26.54	27.51	1.516	0.16	n.s.
2	30.75	28.62	3.656	0.005	†
3	30.43	27.80	4.893	<0.001	‡
	Protein nitrogen				
1	16.84	16.38	1.443	0.18	n.s.
2	17.29	15.91	3.091	<0.01	†
3	16.24	15.25	1.720	0.11	n.s.
	Nonprotein nitrogen				
1	9.70	11.13	2.484	0.03	*
2	13.46	12.71	1.288	0.22	n.s.
3	14.19	12.55	0.439	0.67	n.s.
	CARBOHYDRATE FRACTIONS				
	Reducing sugars				
1	31.68	37.67	19.810	<<0.001	‡
2	53.12	57.96	0.789	0.46	n.s.
3	63.83	74.29	1.399	0.19	n.s.
	Total sugars				
1	86.36	102.52	2.233	0.045	*
2	109.28	119.13	1.246	0.24	n.s.
3	126.70	140.29	1.605	0.14	n.s.
	STARCH-DEXTRIN FRACTION				
1	113.51	158.44	3.504	0.006	†
2	220.59	276.67	4.275	<0.001	‡
3	296.86	348.40	2.921	0.012	*
	READILY AVAILABLE CARBOHYDRATES				
1	199.87	260.96	3.858	0.004	†
2	329.87	395.80	5.751	<0.001	‡
3	423.56	488.69	10.689	<<0.001	‡

n.s. Nonsignificant.
* Significant at 5% level.
† Significant at 1% level.
‡ Significant at 0.1% level.
< Less than.

Discussion

Comparatively few specific data have been gathered on the food reserves of weeds. BARR (5) has summarized the investigations in this field to 1936.

The papers by Arny (1) and Welton, Morris and Hartzler (26) are similar to the study reported in this paper in that they follow the nitrogen and carbohydrate fractions in the roots of perennial weeds, other than bindweed, through the growing season, but the work was not done on material obtained from definite foot intervals of the soil.

Until recently food reserve studies on bindweed have been of a general nature. Barr (5) published the first rather complete analytical study of bindweed reserves in 1936.

It is the intention here to point out certain pertinent findings and viewpoints which may be of value in relating the results reported in this paper to the problem of food reserves in perennial weeds, particularly field bindweed, rather than to compare these with the specific results of other workers.

Nitrogen fractions

Arny (1) states that while nitrogen reserves are present in relatively small amounts in perennial weeds they are of great importance in the metabolism of the plant, particularly at the time of rapid tissue building in the wave of spring growth. Recent studies by Timmons (19, 20, 21, 22, 23), Evans (8), Barr (6) and Bakke et al. (3) indicate that the trend of total nitrogen reserves is independent of the trends shown by the various carbohydrate fractions; that the variation of the nitrogen fractions during the season is low; and that the trend of the total nitrogen fraction has little value as an index to the regenerative power of the plant. Bakke et al. (3) state that their work indicates that nitrogen depletion, unlike carbohydrate depletion, was not directly affected by chlorate treatment.

Carbohydrate fractions

Latshaw and Zahnley (12) made observations in 1927 on the starch reserves of bindweed, and Crafts and Kennedy (7) stated in 1930 that the persistent vegetative activity of the plant is made possible by the storage of relatively large quantities of starch in the root system. Barr (5) published in 1936 the first detailed study of the carbohydrate reserves of the bindweed plant. This study, however, was on material obtained at one sampling date, viz., August 5, 1935. Extensive work has been done on the trend of the carbohydrate fractions in undisturbed bindweed by the following workers on the federal bindweed project: Bakke (4) at Hawarden and Cherokee, Iowa; Evans (8) at York, Nebraska; Seely (16) at Genesee, Idaho; Stahler (18) at Lamberton, Minnesota; and Timmons (23, 24) at Hays, Kansas. A summarized report[3] of their work indicates that the computed fraction, the readily available carbohydrate fraction, has the same general trend at the 5 stations if the time of the start of spring growth and the subsequent stages of growth are taken into consideration. Consisting as it does of the total sugars and the starch-dextrin fraction, this computed

[3] Progress report of cooperative weed investigations. U. S. Dept. Agr., B. P. I., Washington, D. C. 1939. Mimeographed.

fraction was found by these investigators to be the most reliable index of the food reserves of the plant. BAKKE *et al.* (3) state that the reserves of the bindweed roots consist largely of sucrose and a dextrin-like compound or group of compounds. In the study reported herein these substances are included in the fraction designated as the readily available carbohydrates. BARR (6) gives the trends for the carbohydrate fraction in undisturbed bindweed for 3 years' investigations in Colorado. The trends for the starchdextrin fraction and total sugar fraction ran quite similar to those reported in this paper. There was a difference in the reducing sugar trend, however. The total sugar percentages were much higher than those reported in this paper or by TIMMONS (19, 20, 21, 22).

Most of these analytical studies were on bindweed root material taken in the 0- to 12-inch or the 6- to 18-inch soil layer with certain exceptions such as: TIMMONS (21, 22) included five periods of sampling at what he termed "critical points in the seasonal curve for food reserves," taking samples from the 6- to 18-inch, 18- to 30-inch, and 30- to 48-inch soil layers; KIESSELBACH *et al.* (11) made "fodder" analyses of roots taken to a depth of 4 feet; BAKKE *et al.* (3) analyzed samples taken to a depth of 17 feet in 1936 and to a depth of 8 feet in 1937 as well as samples used in routine studies on the 0- to 12 and 12- to 36-inch soil layers. The study reported herein is believed to be the first in which detailed analyses were made on undisturbed bindweed roots from the first, second, and third foot soil levels throughout the growing season.

Summary

1. For the purpose of studying the distribution and seasonal trend of the various organic reserves in the roots during the growing season the root system was arbitrarily divided into the permanent lateral roots and the two types of vertical roots. The two types of vertical roots were each divided into the first, second, and third foot portions, which with the lateral roots gave a total of seven portions. Only roots of well-established, undisturbed plants were used, subject to but little competition from a sparse but uniform stand of other weeds.

2. The percentages of total nitrogen and protein nitrogen fractions in the seven portions of the bindweed root system at fourteen periods of sampling—April 1 to November 1, inclusive—were determined and reported.

3. A fraction obtained by subtracting the protein nitrogen fraction from the total nitrogen fraction was computed and reported. It is designated the nonprotein nitrogen fraction.

4. The general trend of the nitrogen fractions in practically all portions of the plant showed an early season rise to April 15, followed by a decline to a low point sometime during the interval, May 15 to June 15. Marked fluctuations occurred following this interval in many of the portions, succeeded by a second low interval, August 15 to September 1. In certain instances this was the low point of the season of the reserve fraction in that root portion. Additional fluctuations followed, which included a rise on

September 15 followed by a decline and then a rise on November 1.   The general range of percentages was similar in the corresponding portions of the two types of vertical roots, and in the first foot portions of those roots and the lateral roots if the few extreme variations are not allowed to bias the picture.

5. The percentages of reducing sugars, total sugars, and starch-dextrin fraction in 7 portions of the bindweed root system at 14 periods of sampling —April 1 to November 1, inclusive—were determined and reported.

6. A composite fraction, consisting of total sugars and the starch-dextrin fraction, was computed and reported.   It is designated the readily available carbohydrate fraction.

7. All of the carbohydrate fractions, except the reducing sugar fraction, reached low points in all of the root portions during the interval of April 15 to May 15.   In many instances this was the low point of the season for that fraction in that portion.

8. The total sugars attained a seasonal maximum the first of November in the lateral roots and the first foot portion of the two types of vertical roots.   In the other portions of the root system there was a tendency to attain a high point on or before July 15 and a maximum later in the season but prior to November 1.

9. The starch-dextrin fraction attained a maximum in the permanent lateral roots and in the first foot portion of the two types of vertical roots on October 1, and in the interval of August 15 to September 1 in the third foot portion of the vertical roots.   The second foot portion of the vertical roots reached high levels on August 15 and October 1.

10. The readily available carbohydrate fraction followed the trend of the starch-dextrin fraction closely, attaining a maximum in the permanent lateral roots and the first foot portion of the two types of vertical roots on October 1, and in the second and third foot portions of the two types of vertical roots in the interval of August 15 to September 1, except in the second foot of the primary vertical root which attained seasonal high points on August 15 and October 1.

11. The total nitrogen fraction and the total readily available carbohydrate fraction are considered more reliable indices of the reserves of the plant than any of their component parts, the readily available carbohydrate fraction being the more reliable of the two.

12. The differences between comparable portions of the vertical roots were treated statistically.   The t test was used to determine probability. Certain of the differences were statistically significant, some to a high degree. The effect of control procedures on these differences is not known.

13. All results were also computed and reported on a basis of the actual amount in grams per hundred lineal feet of each of the reserve food fractions in each of the root portions.

Dr. E. C. MILLER, Kansas State College, for helpful suggestions during the course of this study.

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LITERATURE CITED

1. ARNY, A. C. Variations in the organic reserves in underground parts of five perennial weeds from late April to November. Minnesota Agr. Exp. Sta. Tech. Bull. 84. 1932.

2. Assoc. Offic. Agr. Chem., Methods of analysis. 5th ed. Washington, D. C. 1940.

3. BAKKE, A. L., GAESSLER, W. G., and LOOMIS, W. E. Relation of root reserves to control of European bindweed, *Convolvulus arvensis* L. Iowa Agr. Exp. Sta. Res. Bull. 254. 1939.

4. —————————. Investigations at Hawarden and Cherokee, Iowa. Progress report of cooperative weed investigations. U. S. Dept. Agr., B. P. I., Washington, D. C. 1939. Mimeographed.

5. BARR, C. G. Preliminary studies on the carbohydrates in the roots of bindweed. Jour. Amer. Soc. Agron. **28**: 787–798. 1936.

6. —————————. Organic reserves in the roots of bindweed. Jour. Agr. Res. **60**: 391–413. 1940.

7. CRAFTS, A. S., and KENNEDY, P. B. The physiology of *Convolvulus arvensis* (morning glory or bindweed) in relation to its control by chemical sprays. Plant Physiol. **5**: 329–344. 1930.

8. EVANS, L. S. Investigations at York, Nebraska. Progress report of cooperative weed investigations. U. S. Dept. Agr., B. P. I., Washington, D. C. 1939. Mimeographed.

9. FISHER, R. A., and YATES, F. Statistical tables for biological, agricultural and medical research. Oliver and Boyd. London. 1938.

10. FRAZIER, J. C. Nature and rate of development of the root system of *Convolvulus arvensis*. Bot. Gaz. **104**: 417–425. 1943.

11. KIESSELBACH, T. A., PETERSEN, N. F., and BURR, W. W. Bindweeds and their control. Nebraska Agr. Exp. Sta. Bull. 287. 1934.

12. LATSHAW, W. L., and ZAHNLEY, J. W. Experiments with sodium chlorate and other chemicals as herbicides for field bindweed. Jour. Agr. Res. **35**: 757–767. 1927.

13. LOOMIS, W. E., and SHULL, C. A. Methods in plant physiology. McGraw-Hill Book Co. New York. 1937.

14. MILLER, E. C. A physiological study of the winter wheat plant at different stages of its development. Kansas Agr. Exp. Sta. Tech. Bull. 47. 1939.

15. MUNSON, L. S., and WALKER, P. H. The unification of reducing sugar methods. Jour. Amer. Chem. Soc. **28**: 663–686. 1906.

16. SEELY, C. I. Investigations at Genesee, Idaho. Progress report of cooperative weed investigations. U. S. Dept. Agr., B. P. I., Washington, D. C. 1939. Mimeographed.

17. SNEDECOR, G. W.   Statistical methods: Applied to experiments in Agriculture and biology.   Iowa State College Press.   Ames, Iowa. 1940.

18. STAHLER, L. M.   Investigations at Lamberton, Minnesota.   Progress report of cooperative weed investigations.   U. S. Dept. Agr., B. P. I., Washington, D. C.   1939.   Mimeographed.

19. TIMMONS, F. L.   Annual report from the bindweed control project. Fort Hays (Kansas) Exp. Sta.   1936.   Unpublished.

20. ——————.   Annual report from the bindweed control project.   Fort Hays (Kansas) Exp. Sta.   1937.   Unpublished.

21. ——————.   Annual report from the bindweed control project.   Fort Hays (Kansas) Exp. Sta.   1938.   Unpublished.

22. ——————.   Annual report from the bindweed control project.   Fort Hays (Kansas) Exp. Sta.   1939.   Unpublished.

23. ——————.   Investigations at Hays, Kansas.   Progress report of cooperative weed investigations.   U. S. Dept. Agr., B. P. I., Washington, D. C.   1939.   Mimeographed.

24. ——————.   Results of bindweed control experiments at the Fort Hays Branch Station, Hays, Kansas, 1935 to 1940.   Kansas Agr. Exp. Sta. Bull. 296.   1941.

25. WEAVER, J. E.   Root development of field crops.   McGraw-Hill Book Co.   New York.   1926.

26. WELTON, F. A., MORRIS, V. H., and HARTZLER, A. J.   Organic reserves in relation to the eradication of Canada thistles.   Ohio Agr. Exp. Sta. Bull. 441.   1929.

SOLUBLE CONSTITUENTS AND BUFFER PROPERTIES OF ORANGE JUICE[1]

E. T. BARTHOLOMEW AND WALTON B. SINCLAIR

(WITH THIRTEEN FIGURES)

Orange fruits undergo certain definite and progressive changes in chemical composition during growth and maturation. The rates at which these changes occur depend chiefly upon climatic and soil conditions, but they are also somewhat affected by the rootstock, as shown by HODGSON and EGGERS (15), SINCLAIR and BARTHOLOMEW (17), and others. While studying the influence of rootstocks on the composition of citrus fruits, it seemed important to the authors to investigate the changes that occur in the soluble constituents of the juice during growth and maturity of the fruit, and, especially, to correlate the concentration of one soluble constituent with that of another for the purpose of determining their physiological and biochemical characteristics. The present studies were therefore undertaken.

It is well known that differences in the composition of citrus fruits are due not only to variety and similar factors, but also to the influence of the geographical location in which the fruits are grown, and to seasonal changes affecting the rate of the ripening processes. A study of the effects of these environmental factors on the composition and quality of the fruit has clearly demonstrated that, without actual investigation, it is not safe to conclude that the characteristics of the citrus fruits in one locality are the same as those of the fruits in another locality. For this reason it is of extreme importance to know the changes in soluble solids, total and reducing sugars, acids, and pH that occur during the growth and maturity of citrus fruits in the different citrus-growing areas of southern California. A knowledge of the interrelation of these particular constituents has served as a foundation upon which a more extended and specialized program of research on citrus fruits has been initiated.

The results reported in the first part of the present paper, which concerns the interrelation of juice constituents, are especially significant because they show the combined effects of different rootstocks, soils, and regional and annual climatic factors on the constituents of orange juice, over a period of several years. Data presented later in the paper show the effect of external factors and of some of the juice constituents on the buffer properties of the juice.

In southern California, navel oranges reach the stage of commercial maturity during a period extending from late November to January; Valencias reach this stage from late March to May, the exact time depending upon the locality in which the fruits are grown. The period of fruit sampling for

the present studies extended for a considerable time both before and after the stage at which the fruit became commercially mature. Such a study shows the changes that occur in the fruit as it grows and matures.

Materials and methods

FRUIT AND JUICE SAMPLES

The major portion of the fruit samples for juice analysis were taken over a period of seven years (1936–1942) from one Valencia grove and one Washington Navel grove at Riverside (inland area) and from one Valencia grove near Tustin (coastal area). These groves had been set out for the rootstock studies previously mentioned. Each of the 14 plots of 10 trees each in the Valencia grove at Riverside represented a different rootstock. The 14 rootstocks were duplicated in the 14 similar plots in the Valencia grove at Tustin. Thirteen of these rootstocks and one other stock were represented in the 14 plots in the Washington Navel grove at Riverside. When the rootstock project was begun, great care was exercised in order to obtain uniform buds and stocks (20).

The remainder of the fruit samples were obtained over a period of four years (1939–1942) from a relatively large number of Valencia- and Washington-Navel-orange groves in coastal, inland, and intermediate areas of southern California. In these groves each sampling plot consisted of at least 25 trees.

In sampling, 6 average-sized fruits were picked from each of the 10 or more trees in each plot. The fruits on some trees were larger than those on others, but only fruits of average size on any given tree were chosen. In some of the tests for determining the accuracy of the method of sampling, the fruits were picked at random; in all other tests, however, 3 fruits were picked from the north side of the tree and 3 from the south side. Samples were selected in this manner because previous investigation (5) had shown that the soluble constituents of fruits from the south side of the tree are higher than those of fruits from the north side and that, as a rule, small fruits have a higher concentration of soluble solids than large fruits.

In all tests the fruits were halved and the juice was extracted from both halves by means of a hand reamer. Both halves of the fruits were used because it is now generally known (5) that there is a higher concentration of soluble constituents in the stylar half than in the stem half of the fruit. The various organic analyses of the juice were made after it had been thoroughly mixed and the portions for analysis had been strained and centrifuged.

The Navel fruits used for determining the buffer properties of orange juice were about three and one-half months beyond the initial stage of commercial maturity; the Valencia fruits were only about two to four weeks beyond this stage. The ratio of soluble solids to acids in the Navel-orange juice was 16.4 : 1; in the Valencia juice, it was 10.8–11.9 : 1.

ANALYSIS OF JUICE

Total soluble solids were determined with an Abbé refractometer, and the refractive indexes were converted to soluble solids by means of a sucrose table. Strictly speaking, therefore, the total soluble solids, as recorded in this paper, are in terms of the percentage concentration which would equal a sucrose solution having the same refractive index. STEVENS and BAIER (19) have shown that refractometer readings for orange juice are too low, mainly because of the citric-acid content of the juice. Since the corrections for orange juice, such as that used in the present experiments, are small (maximum, 0.31 per cent.), none were made. The general conclusions are the same as if the corrections had been made.

Total acidity, expressed as citric acid, was determined by titrating an aliquot portion of the juice with a standard solution of NaOH, with phenol-phthalein as an indicator; all titrations were made in air. All pH values were determined with a Beckman glass electrode pH meter. Titration curves were experimentally determined on samples of juice free of pectin. The pectins were precipitated by pouring the juice (25 ml.) into 95 per cent. ethyl alcohol (100 ml.) and allowing the mixture to stand overnight at room temperature. The pectins were filtered from the solution and washed thoroughly with alcohol. The solution and washings were combined and distilled in vacuum at 55° C. to free the solution of alcohol. The course of the titration curve was determined on this alcohol- and pectin-free solution.

Titration curves were also determined on solutions of citric acid to which the ash from orange juice had been added. The ash was obtained by evaporating to dryness, on a steam bath, known portions of juice (25 ml.) which were subsequently charred and ashed below red heat. The ash was then transferred quantitatively to a beaker and sufficient citric-acid solution was added to make the volume and concentration equivalent to that of the original orange juice.

The sugar determinations were made by the HAGEDORN and JENSEN (12, 13) method as modified by BLISH (6, 7). The strength of the reagents employed by BLISH was satisfactory for determining the total reducing substances (reducing and total sugars as glucose) in unclarified orange juice when the values ranged from 3 to 10 mg. in 10 ml. of diluted citrus juice. The samples were diluted so that the values fell within this range. This method was used because comparative tests showed that it was more rapid than the best of the copper reduction methods and, at the same time, gave comparable results for the quantity of sugar in the sample. The standard HCl method was used to invert the sucrose. After adding the HCl, the solution was allowed to stand at room temperature (approximately 22° C.) for 24 hours before making the determination. Parallel tests showed that HCl was as reliable as invertase scales (Wallerstein's) for inverting the sucrose in orange juice. The sugar values presented in this paper are probably a little high because the samples of juice were not clarified before the sugar determinations were made. Samples of unclarified juice showed a

reducing-sugar content of approximately 6.50 per cent. and a total-sugar content of approximately 12.54 per cent., while the corresponding values for clarified samples of the same lot of juice were 6.36 per cent. and 12.26 per cent. The comparatively small increase in accuracy to be gained did not appear to justify the expenditure of the extra time and labor that would have been necessary to clarify each sample of juice. The glucose factor on the reagents was determined with a sample furnished by the National Bureau of Standards. The acid and reducing-sugar determinations were made within 3 hours, usually within 30 minutes, after the fruit was reamed.

Results

THE SOLUBLE CONSTITUENTS IN THE JUICE

Since 80 to 85 per cent. of the oranges that reach the commercial channels of distribution are finally consumed as juice, it follows that the com-
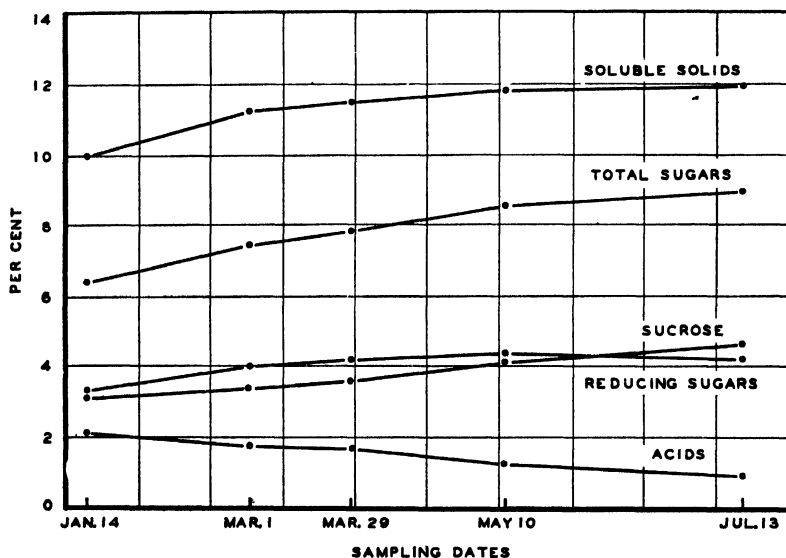
FIG. 1. Changes in percentages (fresh-weight basis) of soluble solids, total sugars, sucrose, reducing sugars, and acids of Valencia-orange juice with the advance of the season (January 14 to July 13). Each of the first four points on each curve represents the mean of 28 determinations made in 1939, on the date specified; the fifth point on each curve represents the mean of 72 determinations made on July 13 of three consecutive years, 1936, 1937, and 1938.

position of the juice is more important, in relation to fruit quality, than the composition of the whole fruit. This fact has been recognized for many years by investigators in the citrus industry (3, 9), and it has served as a basis for formulating the legal maturity test (ratio of soluble solids to acids) in use at the present time. In general, the ratio of soluble solids to acids in the fruit juice may be used as a test for maturity because the total soluble solids increase, and the acids decrease, during growth and maturation of the fruit.

The data illustrated in figure 1 indicate the truth of the foregoing state-

ment in that the total soluble solids and the reducing and total sugars increased, while the titratable acidity decreased, at approximately the same rate, until May 10. After that date the soluble solids and total sugars increased more slowly and the sucrose and acids decreased. The reducing sugars alone continued to show the usual upward trend. These curves represent only the means of a large number of determinations. Later in this paper it is shown that there may be wide divergences from the means shown in figure 1. Although the curves were constructed from data based on determinations on Valencia-orange juice, Navel-orange juice was found to show the same trends. Each of the first four points on the curves (fig. 1) represents the mean value of 28 determinations obtained in 1939 from fruit from the Valencia plots located at Riverside and at Tustin. The fifth point on each curve represents the mean value of 72 determinations obtained from
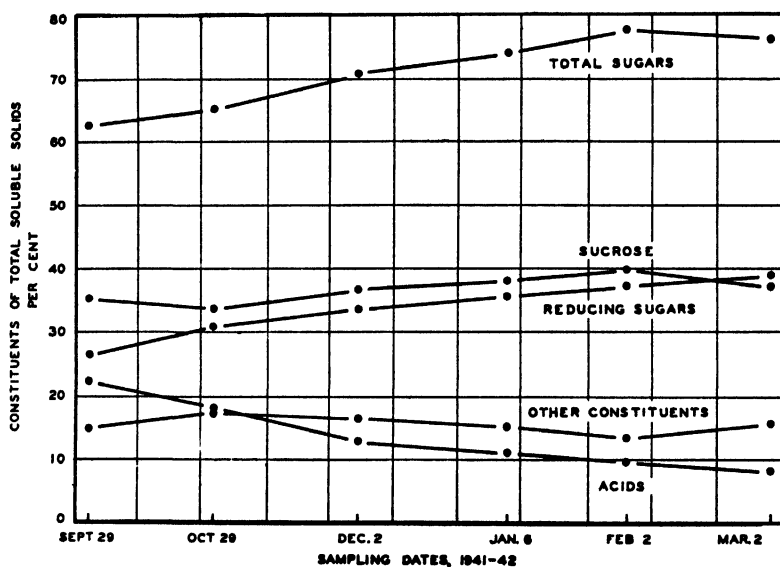
FIG. 2. Concentration of total sugars, sucrose, reducing sugars, acids, and other constituents in Navel-orange juice, expressed as percentages of total soluble solids (dry-weight basis), determined on samples picked at different times during the season. The maturity of the fruit ranged from approximately three months before the initial stage of commercial maturity to three months after.

the fruit from trees on the same Riverside and Tustin plots over a period of three consecutive years, 1936, 1937, and 1938.

The relative percentages of the different soluble constituents, based on total soluble solids, found in Navel-orange juice, at intervals from September 29, 1941, to March 2, 1942, are shown in figure 2. Total sugars increased from about 63 per cent. to a maximum of about 77 per cent. on February 2. The reducing sugars steadily increased over the entire period, from about 27 per cent. to about 38 per cent. Except for a drop on October 29, the sucrose increased from about 36 per cent. on September 29, to 40 per cent. on February 2. By March 2 it had dropped to about 37 per cent. Both of the

decreases in sucrose appear to be registered in the total-sugar values for these dates (October 29 and March 2). The acids decreased from about 23 per cent. to about 8 per cent. of the total soluble solids. The fraction of soluble solids designated as "other constituents," consisting of inorganic compounds, amino acids, ascorbic acid, a small amount of soluble pectins, etc., remained nearly uniform throughout the sampling period. The relative percentages of soluble constituents in Valencia-orange juice are similar to those in Navel-orange juice. The total sugars in the juice of fully mature Navel and Valencia oranges may comprise at least 75 to 80 per cent. of the total soluble constituents.

A comment should be made about the basis upon which the calculations have been made in figures 1 and 2. In the former, the constituents were calculated on the fresh-weight basis of the juice; in the latter they were cal-
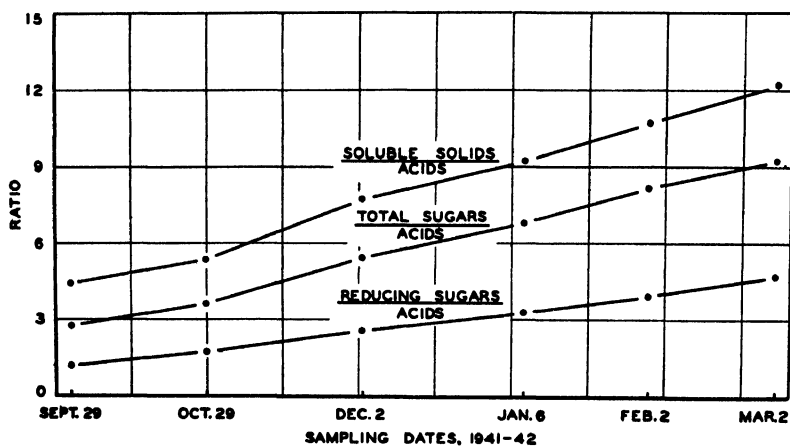
FIG. 3. Ratios of total soluble solids, of total sugars, and of reducing sugars to acids in Navel-orange juice, determined on samples picked at different times during the season. The maturity of the fruit ranged from approximately three months before the initial stage of commercial maturity to three months after.

culated on the dry-weight basis. It may be mentioned here that the figures for the percentage of total soluble solids in orange juice (fresh-weight basis) agree very closely with those for the dry weight of a sample of the same juice (5).

Since the total soluble solids, total sugars, and reducing sugars increased during the growing season, it is of importance to know the rate of change of these constituents with the change in concentration of acid. This has been graphically illustrated by plotting the ratios of these substances to total acidity against the time of sampling (fig. 3).

The ratio of soluble solids to acids serves, in part, as an index to maturity, for commercial purposes. It is difficult to attribute any great physiological significance to this ratio, however, as it is easily affected by very small changes in the acid concentration of the juice. Most of the soluble constituents in the juice are sugars, and this particular ratio is therefore often

referred to as the sugar-acid ratio. This usage is questionable, however, since the two ratios are very different, numerically and physiologically, chiefly because, as shown in figure 2, 10 to 15 per cent. of the soluble solids are not sugars and acids. Of interest is the fact that on September 29 the nonsugars included approximately 38 per cent. of the total soluble solids, while on March 2 they included only 24 per cent. (fig. 2). Practically all of this difference, however, was caused by the decrease in acids.

Although the ratio of total sugars to acids is not so high as that of soluble solids to acids, the rate of change during the season is about the same (fig. 3). The proportion of reducing sugars to acids shows a lower but similar relationship. These three ratios further demonstrate the inverse relation between the concentrations of soluble solids and sugars and the concentration of acids in the fruit, which signifies that, with increase in soluble solids and sugars, a corresponding decrease in concentration of acids occurs during the period of growth and maturation.

RELATION OF TOTAL SOLUBLE SOLIDS TO TOTAL SUGARS, REDUCING
SUGARS, AND ACIDS IN THE JUICE

Since a large number of determinations on the juice have been made in these studies, sufficient data are available to illustrate important and worth-
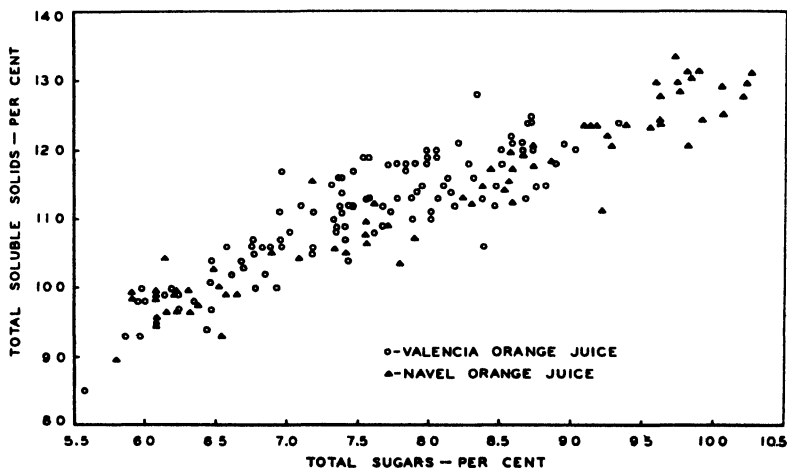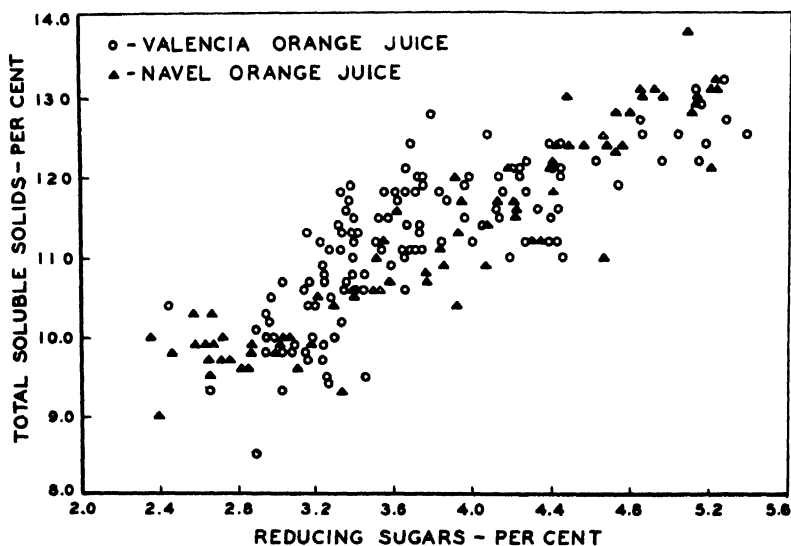

FIG. 4. Scatter diagram showing the relation of total soluble solids to total sugars in juice of Valencia and Navel oranges. Fruit maturity ranged from immature (green) to very mature (Valencias picked January 14 to July 13, 1939; Navels, September 29, 1941, to March 2, 1942).

while correlations of total soluble solids with certain other soluble substances in the juice.

In figure 4 the percentages of total soluble solids are plotted against percentages of total sugars of different samples of juice of Valencia and Navel oranges. The wide range shown (fig. 4) in concentration of total sugars, represents the amounts of sugar that occurred in fruits ranging from immature to fully mature. It is evident that within this range of fruit develop-

ment, the increase in total sugars parallels that of total soluble solids. These two variables should be closely correlated for the total sugars represent, on the average, from 63 to nearly 80 per cent. of the total soluble solids during the period over which this study was made (fig. 2). This is further evidence that soluble solids and total sugars, as already shown in figure 1, have a tendency to increase at about the same rate. There is sufficient scattering of the points (fig. 4), however, to show that the juice may contain 11.0 per cent. soluble solids, and that the total sugar content may range from 7 to 9 per cent.; or that, on the other hand, juice containing 8.5 per cent. total sugars may range from 10.5 to 12.5 per cent. soluble solids.

The concentration of reducing sugars in orange juice amounts to somewhat less than one-half of the total sugars, and the ratio remains fairly con-
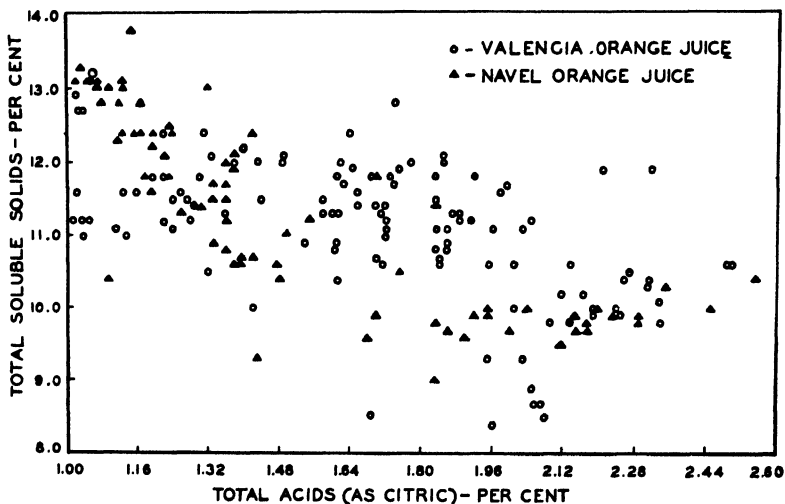

FIG. 5. Scatter diagram showing the relation of total soluble solids to reducing sugars in juice of Valencia and Navel oranges. Fruit maturity ranged from immature (green) to fully mature (Valencias picked January 14 to July 13, 1939; Navels, September 29, 1941, to March 2, 1942).

stant during the development and maturation of the fruit. It follows then that the relation between total soluble solids and reducing sugars (fig. 5) should be similar to that between total soluble solids and total sugars. Experimentally, however, the points for the values of reducing sugars showed a greater scattering on the diagram than those for total sugars. To illustrate (fig. 5), orange juice containing 11.0 per cent. total soluble solids may have reducing sugars ranging anywhere from approximately 3.2 to 4.5 per cent.; or, conversely, juice having reducing sugars of approximately 3.4 per cent. may contain total soluble solids of from 9.25 to 12.0 per cent. This may have been partly due to undetermined changes in the juice during the lapse of time between its extraction and analysis. Since in nearly all cases the reducing sugars were determined within 30 minutes after the juice was

extracted, it does not seem that this could have been an important factor. Part of the excessive scattering of the points for the reducing sugar values is probably due to the fact that this carbohydrate fraction is quickly and easily used in metabolic processes. From limited data obtained during the course of this experimental work, it appears that, under certain weather conditions, the concentration of reducing sugars is more variable than is that of nonreducing sugars.

It has already been noted that the sugars increase and the acids decrease in orange juice during growth and maturity of the fruit. The same trend prevails between total soluble solids and total acidity. Nevertheless, it is clear from the data in figure 6 that large changes can occur in total acidity

FIG. 6. Scatter diagram showing the relation of total soluble solids to total acids in juice of Valencia and Navel oranges. Fruit maturity ranged from immature (green) to fully mature (Valencias picked January 14 to July 13, 1939; Navels, September 29, 1941, to March 2, 1942).

with only a slight change in total soluble solids. The reverse is also true. This wide range in acid concentration (1 to 2.6 per cent.) shows that the juice samples were from fruits ranging from immature (green) to fully mature. These data explain why in many instances high concentration of soluble solids at maturity may not necessarily be correlated with a correspondingly low concentration of acids. Although it is generally true that the acids decrease and the soluble solids and sugars increase as the season advances, large variations in soluble solids and sugars of the juice frequently occur with only slight changes in total acids. For example, figure 6 shows that orange juice containing 11.0 per cent. total soluble solids may have a total acidity ranging anywhere from 1.00 to 2.60 per cent.; or, conversely, that juice having a total acidity of approximately 1.75 per cent. may contain anywhere from 8.5 to 13.0 per cent. total soluble solids. Results of this kind indicate the chances for error in using the ratio of soluble solids to

acids as the sole criterion of maturity of fruit and quality of orange juice. Slight changes in the percentage of acids result in large changes in the ratio without markedly affecting the quality of the juice. The practical aspects of these problems have long been recognized by the industry.

It may be well to point out here that the results shown in figures 1 and 7 appear to be at variance with the results obtained by CALDWELL (8). He reported that in oranges and grapefruit there is a progressive increase in both active and titratable acidity as growth advances toward maturity, but that as ripening begins, both active and titratable acidity start to decrease, and continue to decrease as ripening and maturity proceed. From a study of the two sets of data, it appears that CALDWELL terminated his experiments at about the same stage of fruit-ripening as that at which our experiments began; therefore, a direct comparison of the two sets of results cannot be made.

RELATION OF pH TO TITRATABLE ACIDITY OF ORANGE JUICE

pH AND TOTAL ACIDITY.—Juices of fruits and vegetables may have a comparatively low or a comparatively high total acidity and yet have approximately the same pH value, the condition depending upon the kinds and amounts of buffer salts present. To illustrate with an actual example, juice from Navel oranges grown on Rough-lemon rootstock, showed 0.80 per cent. total acids (in terms of citric acid), with a pH of 3.52, while juice from Navel oranges grown on Trifoliate orange showed 1.21 per cent. total acids, with a pH of 3.46. Although there was a 34 per cent. difference in the total acidity of these two juice samples, the pH values differed only slightly. These two samples of Navel oranges were picked on the same day (March 3, 1942) from two different blocks in the same grove.

During the course of this investigation, it was necessary to make a large number of determinations of pH and of total acids (titratable acidity), on juice of oranges from samples collected at various stages of maturity. These data, as represented in figure 7, show the relation between pH value and titratable acidity. It can be observed that these pH values were determined over a wide range of total-acid concentration (0.7 to 2.60 per cent.). Juice samples containing 2.60 to 0.70 per cent. total acids, represent fruits ranging from immature (green) to fully mature.

The data reported here (fig. 7) show that the pH value of orange juice bears a definite relation to the titratable acidity, if compared over a wide range of acid concentration. This relationship, however, is not so definite over shorter ranges of acid concentration, within which two samples of orange juice having rather large differences in total acidity may have the same pH value. For example, orange juice with a pH value of 3.20 may have a total acidity ranging from approximately 1.10 to 1.85 per cent.; or, conversely, juice with a total acidity of approximately 1.43 per cent. may yield pH readings ranging from 3.00 to 3.43. Under these conditions, the pH value does not indicate the amount of acid present, nor does a given acid value represent a definite pH value.

TITRATION CURVES OF ORANGE JUICE.—The relation of pH to titratable acidity (total acids) of orange juice can be further elucidated by following its resistance to changes in pH upon addition of increments of standard alkali to known amounts of orange juice. Orange juice, like all plant ex-
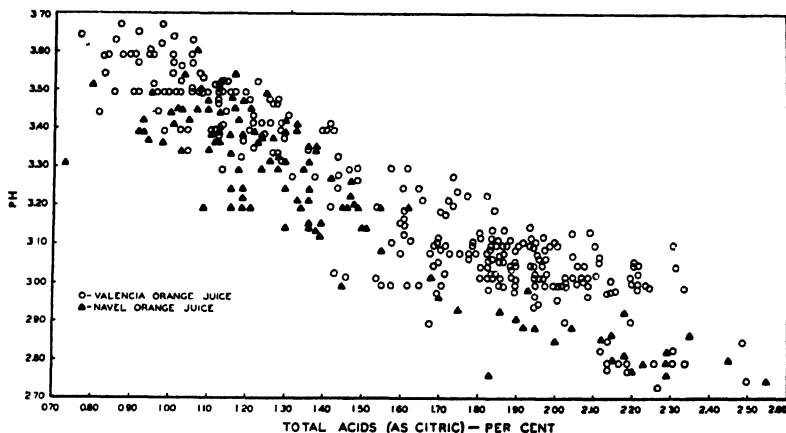

FIG. 7. Changes in pH and total acidity (titratable acidity) in juice of Valencia and Navel oranges as the season advances. As shown in figures 4, 5, and 6, a given value in one variable may represent many values in the other. Fruit maturity ranged from immature (green) to fully mature (Valencias picked January 14 to September 5, 1939; Navels, September 29, 1941, to March 25, 1942).

tracts, is, to a varying degree, capable of resisting changes in pH upon the addition of strong acids or bases. It was therefore decided to determine experimentally what substances were responsible for the buffer effect of the juice. The results of these experiments are given in the following paragraphs.
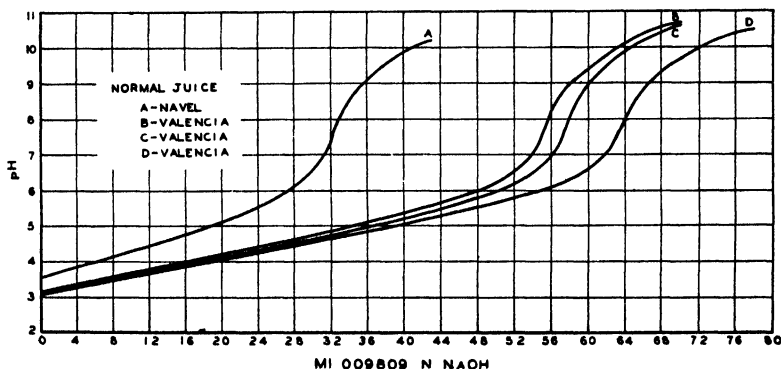
FIG. 8. Titration curves of various samples of Navel- and Valencia-orange juice containing different amounts of total acids. The ratio of soluble solids to acids was 16.4 : 1 for Navel juice, and 10.8–11.9 : 1 for Valencia juice.

An inspection of the buffer curves of different samples of normal orange juice (fig. 8) shows that the four samples did not contain the same concentration of acid. The endpoints calculated from curves A, B, C, and D occurred at pH 7.82, 7.81, 7.85, 7.83, respectively, but the differences in milli-

liters of 0.09809 N NaOH necessary to arrive at the pH of the endpoint show the differences in the amount of acid in the juices. If these juices, which were from fruits from different trees in the same grove, had possessed the same concentration of acid, all four of the curves would have been very close together.

In the course of this study, it was important to determine the effect of the soluble pectins in the juice on the shape of the buffer curve. The titration curves of orange juice from which the pectins had been removed, and of normal orange juice, are shown in figure 9, A and B, respectively. These two curves are very close. The slight difference between them may be partly due to the fact that a very small amount of a volatile acid was distilled with the alcohol during vacuum distillation, and partly due to the lack of pectin, which reacts with NaOH beyond pH 7. The course of the buffer curve of
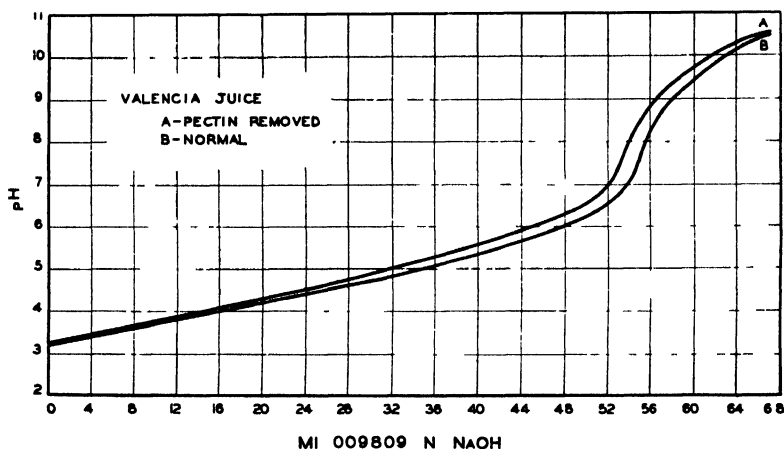
FIG. 9. The effect of pectins on the buffer action of Valencia-orange juice: A, pectins removed; B, normal. Both samples were from the same lot of juice, in which the ratio of soluble solids to acids was 10.8: 1.

the pectin-free juice definitely shows, therefore, that these pectin-free samples required less alkali for neutralization and that they apparently contained slightly less acid than those of normal juice.

Other titration curves were determined on normal juice only (fig. 10, C); on aqueous solutions of citric acid of a concentration equivalent to the amount calculated from the neutralization point on the curve of the normal juice (fig. 10, B); and, finally, on citric-acid solutions, with total acidity equivalent to that of the juice, plus the ash elements from a 25-ml. portion of the normal juice (fig. 10, A). A comparison of the buffer curve of citric acid (fig. 10, B) with that of normal Valencia-orange juice (fig. 10, C) shows that citric-acid solution has less buffering effect than orange juice. It is interesting to note, also, that the pH of the citric-acid solution is much lower than that of the juice. On the addition of ash elements to the citric-acid solution (fig. 10, A), the pH immediately increased from 2.07 to 3.10, the latter value being nearly the same as that of the original juice. This was
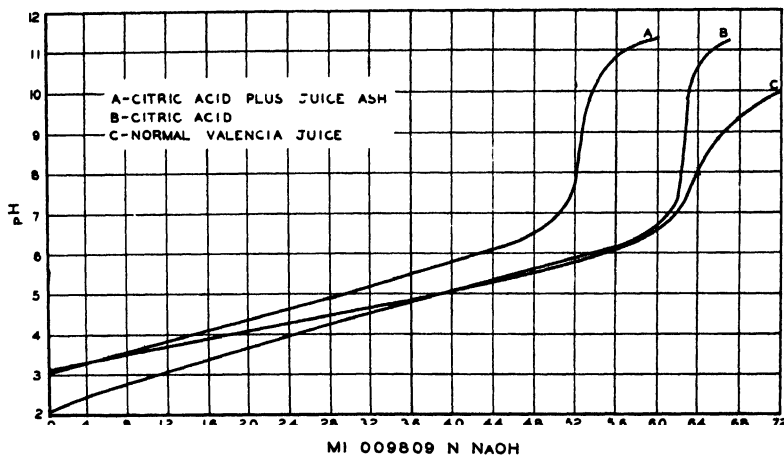
FIG. 10. Comparison of the buffer properties of (A) pure citric acid plus the ash elements from a 25-ml. portion of Valencia-orange juice, (B) pure citric acid, and (C) normal Valencia-orange juice. The ratio of soluble solids to acids for the normal Valencia juice was 11.2 : 1.

caused by the reaction of the citric acid with the base constituents of the ash.

The titration curves of normal Valencia juice and of citric acid containing sodium citrate are shown, for comparison, in figure 11. The concentration of the citric-acid solution (25 ml.) used was equal to the total acidity calculated from the endpoint of the orange-juice curve. As usual, the
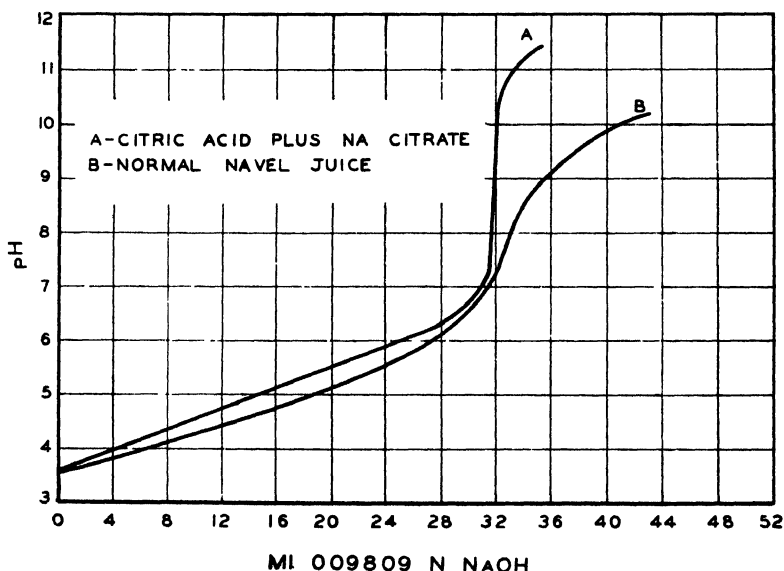
FIG. 11. A comparison of the buffer properties of citric-acid solution and Navel-orange juice: A, pure citric acid (of the same concentration as that of normal Navel-orange juice) to which had been added enough sodium citrate to bring the acid solution to the same initial pH value as that of the orange juice; B, normal Navel-orange juice. The ratio of soluble solids to acids for the normal Navel juice was 16.4 : 1.

initial pH of the acid solution (fig. 11, A) was lower than that of the juice (fig. 11, B). Before the titration was started, a few drops of sodium citrate solution were added to bring the acid solution to the same pH value as that of the juice. The course of the titration curve of the acid solution differs
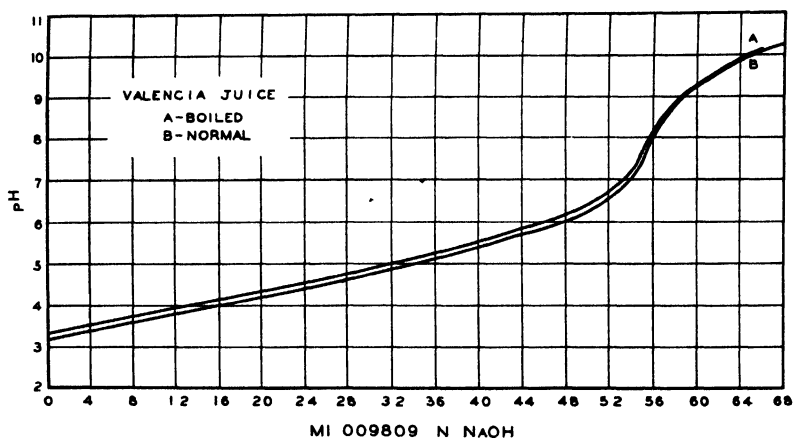

FIG. 12. The effect of heating on the buffer capacity of Valencia-orange juice: A, juice boiled for 45 minutes; B, not boiled. Both samples were from the same lot of juice, in which the ratio of soluble solids to acids was 11.9:1.

only slightly from that of the normal juice up to pH 7; but, for some undetermined reason, beyond this point they show considerable divergence.

The effect of heat on the buffer system of orange juice (fig. 12) is shown by the titration curves for normal Valencia juice (fig. 12, B) and for a portion of the same juice boiled for 45 minutes (fig. 12, A). The boiling of
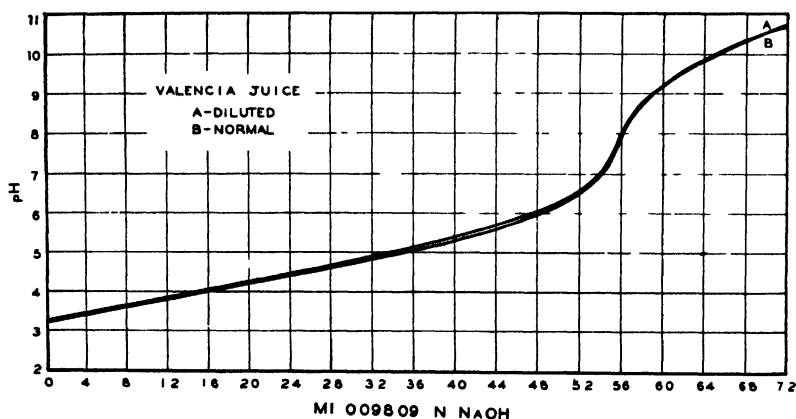

FIG. 13. The effect of dilution on the buffer properties of Valencia-orange juice: A, diluted (1 part juice to 4 parts distilled water): B, undiluted. Both samples were from the same lot of juice, in which the ratio of soluble solids to acids was 10.8:1.

the juice apparently drove off a slight amount of a volatile organic acid or CO_2 to give a slight increase in pH and a slight lowering of the titratable acidity. These small differences were not sufficiently large to cause significant changes in the values or to alter the shapes of the curves.

The effect of heat on the small amount of soluble pectins in the juice was not reflected in these results. The boiling of pectin solution has the tendency to raise the free acidity of the solution slightly and to result in a certain amount of demethylation. In the systems reported here, factors other than pectin were in effect, for there occurred a slight loss, rather than an increase, in acidity.

The high degree of resistance to changes in pH is shown by the fact that orange juice can be greatly diluted without changing the pH or the shape of the titration curve (fig. 13). Although the limits of dilution were not reached in these experiments, titration curves are shown for normal orange juice (fig. 13, B) and for juice diluted in a ratio of 1:4 (fig. 13, A). This partially explains why the concentration of soluble constituents of orange juice can undergo relatively large changes during the growth and maturity of the fruit, with relatively small fluctuations in pH.

Discussion

In order to determine whether 6 fruits from each of the 10 trees in each plot were enough to give reliable results, two tests were made. In the first test, samples of 6, 12, and 18 fruits were picked (half of each sample from the north side of the tree and half from the south side) from each of the 10 trees in one of the plots in the Valencia grove at Riverside. The 6-fruit samples were combined to form sample 1 (60 fruits); the other samples were similarly combined to form sample 2 (120 fruits) and sample 3 (180 fruits). All fruits in each of the three samples were juiced. The percentages of total soluble solids in the juice from samples 1, 2, and 3, were 13.12, 13.12, and 13.07, respectively, a range of 0.05 per cent. In the second test, samples of 6 fruits and 40 fruits (samples 4 and 5, respectively) were picked from each of 10 trees in another plot in a different part of the same grove. All 60 fruits in sample 4 were juiced, but the juice for sample 5 was obtained from only 100 of the 400 fruits in the sample. The 100 fruits were taken at random, by a blindfolded person, from the 400 fruits after they had been thoroughly mixed. The percentages of total soluble solids in the juice from samples 4 and 5 were 12.97 and 12.84, respectively, a difference of 0.13 per cent. The differences in the values shown in these two experiments (0.05 and 0.13 per cent.) were found to be statistically insignificant.

The results of the two preceding tests were confirmed by still another test, in which 24 average-sized mature fruits were selected (12 from the north side of the tree and 12 from the south side) from each of 10 Valencia-orange trees in a different grove, from which no fruits had been picked during the current season. The 24 fruits from tree 1 were segregated at random into four groups (a, b, c, and d) of 6 fruits each. The 24 fruits from each of the other 9 trees were similarly segregated. By this method, each of the combined groups (a, b, c, and d) contained 60 fruits, 6 fruits from each of the 10 trees, as in our regular method of sampling. The total soluble solids for each group of 6 fruits from each tree were determined

separately, however; a total of 40 determinations. The mean percentages and standard errors of means for the total soluble solids were then calculated for each of the four groups of 10 determinations and were found to be as follows: group a, 13.36 ± 0.166; group b, 13.39 ± 0.176; group c, 13.26 ± 0.173; and group d, 13.29 ± 0.146. The difference between the means of the highest and lowest values is only 0.13 per cent., which is statistically insignificant, as shown by the t test at the 5 per cent. level and 18 degrees of freedom (18).

The results obtained in the plot method of sampling used in these tests, should not be given the same interpretation as those obtained by DENNY (10) and by APPLEMAN and RICHARDS (1), who made single-tree determinations. The results shown in the two preceding paragraphs indicate that the general method used in these experiments, in which 6 fruits were selected from each tree, were enough to give a representative sample from each plot of 10 or more trees.

Further comments should be made on the data of figures 1 to 7. Figures 1 and 2 illustrate the types of curves usually constructed to show changes that occur in the composition of fruits during growth and maturity. As only mean values are reported, the information revealed is general and satisfactory for showing the comparative seasonal trends in fruit composition. The curves fail, however, to yield information about variability between different constituents, and about the ranges in concentrations of various soluble solids in the juice during this same period of growth. Some of these relationships are clearly shown in figures 4 to 7, inclusive. It can be observed that, as in most biological data, the total sugars, reducing sugars, and acids in the juice do not bear a linear relation to the total soluble solids. Attention has already been drawn to the variations in amounts of these constituents with the advance of the season. The scattering of the points on the diagrams (figs. 4, 5, and 6) demonstrates the differences in the relative proportions of soluble constituents in the juice. Although the trend of the curves supplies enough information for the formulating of certain generalizations, the dispersion of the values is so great that this should be considered before making such generalizations. The condition is well illustrated by the changes in total soluble solids, with corresponding changes in total acidity, shown in figure 6. In this case, it can be seen that, with a given concentration of either constituent, there results a wide fluctuation in ratios.

The data for figure 4 came from the rootstock plots. In order to determine whether such conditions apply to southern California in general, a large number of similar determinations were made on fruit from groves chosen at random in coastal, inland, and intermediate areas. A scatter diagram of these results has not been included because of the already large number of figures. The data, however, portrayed a type of scattering that was identical with that shown in figure 4. The same comments apply to figures 5 and 6.

Although there is a more or less marked degree of scattering of the points

in figures 4 to 6, the degree of closeness with which any two of the soluble constituents may be related is more definitely expressed by their correlation coefficients, which have been calculated and are shown in table I.

The highest correlation coefficients obtained were those between total soluble solids and total sugars in the juice of fruit of Washington-Navel- and Valencia-orange varieties of the inland area (table I). The correlation of these constituents in juice of Valencia-orange fruits from the coastal area was lower in value. As shown in figure 6, the increase in percentage of total soluble solids and the decrease in percentage of acids do not occur at a uniform rate during the season. This is further emphasized by the significant but low negative correlations between these two variables, in samples

TABLE I

CORRELATION COEFFICIENTS SHOWING THE DEGREE OF CORRELATION OF VARIOUS
SOLUBLE CONSTITUENTS IN ORANGE JUICE

FACTORS CORRELATED	VARIETY OF ORANGE	GROVE LOCATION, SOUTHERN CALIFORNIA	NUMBER OF SAMPLES n	CORRELATION COEFFICIENT* r
Total soluble solids and total sugars	Washington Navel	Inland area	79	+ 0.9700
	Valencia	Inland area	72	+ 0.9219
	Valencia	Coastal area	56	+ 0.8024
Total soluble solids and total acids	Valencia	Inland area	56	− 0.5390
	Valencia	Coastal area	56	− 0.5555
	Valencia	Random†	21	− 0.4386
Total soluble solids and reducing sugars	Valencia	Inland area	56	+ 0.8159
	Valencia	Coastal area	56	+ 0.7096
Total sugars and reducing sugars	Valencia	Inland area	56	+ 0.9028
	Valencia	Coastal area	56	+ 0.8344

* These correlation coefficients are significant, as shown by the t test (18).
† Commercial groves in coastal, inland, and intermediate areas.

from different locations. Very high correlations existed between total sugars and reducing sugars during the period in which these samples were taken, the fruit samples of the Valencia variety from the coastal area yielding a lower correlation than those from the inland area. Data are also reported on samples from groves chosen at random in different citrus-growing areas of southern California. These data are included (table I) for purposes of comparison, in order to demonstrate that these experimental results are applicable in principle to fruit grown for commercial purposes.

The correlation coefficient for the relation of pH to total acids (fig. 7) was not calculated, but the degree of scattering of the points is such that a significant negative correlation is definitely indicated.

The relation of pH to the titratable acidity of orange juice is important in that it is, in general, related to fruit maturity; it is important, also, in that large changes in acid concentrations can occur with only very slight

changes in pH (fig. 7). This characteristic of orange juice is exhibited to a greater or lesser degree by most acid fruits. A quantitative measure of this characteristic may be obtained by observing the change in pH value of the juice upon the addition of increments of standard NaOH, as reported elsewhere in this paper. An inspection of figure 7 shows that at a given pH value, the juice may have various concentrations of total acids. If a range of acid concentration is chosen, for example, a range of 0.70 to 1.00 per cent., such as often occurs in fruit that is commercially mature, it is difficult to detect a direct relation between pH and total acidity. Over the total range of acid (0.70 to 2.60 per cent.), shown in figure 7, the pH tends to decrease with an increase in concentration of acid. In this particular instance, the relation of pH to titratable acidity depends upon the acid range. Other investigators with other fruits have found varying degrees of correlation between these two variables. In studies with 33 varieties of mature Minnesota apples, BARNES (4) obtained a high correlation coefficient ($r = 0.9265$) between titratable acidity and hydrogen-ion concentration of the juice; with 11 varieties of mature grapes, a high correlation ($r = 0.9213$) was also obtained; with 11 varieties of plums at different stages of maturation, the correlation ($r = 0.6198$) was much lower. ASKEW (2), on the other hand, did not find a definite relation between pH and titratable acidity (total acids) of mature apple juice.

The following information is of interest in that it indicates the differences in the proportionate amounts of reducing sugars and sucrose that may exist in orange juice as the season advances. As shown in figures 1 and 2, the reducing sugars comprised *less* than half the total sugars up to the time of making the last determinations—July 13 for Valencias (fig. 1) and March 2 for Navels (fig. 2). On these dates the reducing sugars amounted to *more* than half the total sugars. That this reversal was not a matter of chance is indicated by the fact that, when determinations were made on the juice of Valencia oranges from the 14 different rootstocks in the inland area (Riverside), on September 14 and 15, 1942 (approximately five and one-half months after the fruit had become commercially mature), the reducing sugars, in every case, amounted to more than half the total sugars. The average for the 14 determinations was 5.76 per cent. reducing sugars and 4.75 per cent. sucrose (by difference). The largest and smallest differences were 2.20 and 0.48 per cent., respectively. The results of determinations made on the juice of Valencias on the same kind of rootstocks in the coastal area (Tustin), on September 22, 1942 (approximately four and two-thirds months after the fruit had become commercially mature), were similar, but the differences were not so great. The averages were 5.00 per cent. for reducing sugars and 4.55 per cent. for sucrose. In two of the determinations, however, the percentages for reducing sugars (4.65 and 4.72) were about the same as those for sucrose (4.66 and 4.88), or were a little less. These results, which show a tendency toward increase in reducing sugars and decrease in sucrose in Valencia-orange juice as the season advances, are in line with the results

obtained by HILGEMAN and SMITH (**14**) on grapefruit juice, although the ratios of reducing sugars to sucrose in the grapefruit juice were higher than those in the Valencia juice.

The titration curve of orange juice shows that the buffer system is governed largely by organic acids and by salts of the inorganic constituents in the juice. Hydrolyzed ascorbic acid would furnish acids that would have a buffering effect, but the total acidity in orange juice is due mostly to citric acid. The small amount of soluble pectins has little if any effect on the buffer capacity of the juice, though pectin reacts with NaOH beyond pH 7 to form sodium pectate. Even in weak acid solution, pectins will form a loose combination with cations from neutral salts of calcium, sodium, and potassium. In alkaline solution, considerable time is required for pectin to react completely with NaOH to form sodium pectate. Soluble nitrogen compounds, in the form of amino acids and amides, exists in orange juice in such small amounts that they are of only slight importance in the buffer system. The dissociation constant of sugars is of such magnitude (of the order of 10^{-11}) that the sugars were probably without buffer effect in the present experiments. Regardless of the small amount of buffering that may have been contributed by these and by other substances such as flavonols and acid phosphates in the juice, or by slight ion-activity changes which may have occurred, citric acid and its basic salts, and perhaps some carbonate, play the chief rôle in this phenomenon. This is made evident by the fact that orange juice contains considerable amounts of inorganic salts, as represented by the percentages of ash in the juice (approximately 3.06 per cent. in Valencias and 3.27 per cent. in Navels, on a dry-weight basis). It is not known in what form these salts exist in the juice. Some of the cations undoubtedly are combined with the pectins, even in acid medium, and some are combined with the existing phosphate, sulphate, and other anions that are present; but most of the cations are probably combined with citric acid in the form of citrates. These inorganic citrates and citric acid are the sources of the substantial buffer capacity of orange juice.

The titration curve (fig. 12) for boiled orange juice was determined for the purpose of measuring the effect of heat on the buffer capacity of the juice. This type of experiment should show whether the heat decomposed or volatilized any of the acid constituents and thus lessened the amounts of NaOH required for neutralization. It should show, also, the effect of heat on the soluble pectins or on any other constituent that might hydrolyze and thus increase the amounts of NaOH required for neutralization. In the present experiments, the course of the titration curve for the orange juice that was boiled for 45 minutes (fig. 12, A), was similar to that for normal juice (fig. 12, B). The slightly higher pH value of the boiled juice showed that this juice required slightly less NaOH than unboiled juice for neutralization. Titration curves were also determined on juice boiled for 5, 10, 15, and 30 minutes, respectively; the values were so close to those of normal juice, however, that it was impractical to put them on the graph (fig. 12).

These results agree with those of DUNNE (11), who showed that boiled and filtered sap of wheat and buckwheat plants gave titration curves identical with those of the fresh sap; of BARNES (4), who observed that the hydrogen-ion concentration was only slightly lower in cooked apple juice than in fresh; and of MARSH (16), who showed that the buffer capacity of juice of nonacid vegetables was affected only slightly when heated in acid solution.

Summary and conclusions

The percentages of total soluble solids and of total sugars increased and that of acids decreased at approximately the same rate in the juice of Washington-Navel- and Valencia-orange fruits during maturation. After the fruits reached the stage of commercial maturity, the percentages of total soluble solids, total sugars, and reducing sugars in the juice continued to increase, while the sucrose and acids decreased, as the season advanced. The rate of increase in total soluble solids, however, was not so great as that of the total sugars and reducing sugars.

From September 29 (fruit still green) until March 2 (fruit well beyond the initial stage of maturity), the soluble solids of Washington-Navel-orange juice in California are composed of approximately 63 to 77 per cent. total sugars (35 to 40 per cent. sucrose and 27 to 38 per cent. reducing sugars), 23 to 8 per cent. acids, and 15 per cent. other substances. The values for Valencia juice of corresponding maturity are similar.

Where individual values for total soluble solids were plotted against the corresponding values for total sugars, noticeable scattering of the points occurred, but an increase in total soluble solids generally involved a corresponding increase in total sugars. This was verified by the high correlation coefficients calculated between total soluble solids and total sugars. A similar relation between total soluble solids and reducing sugars was noted, but a considerably greater scattering of the points occurred.

Although the total acids decreased during the growth and ripening of the fruit, while total soluble solids increased, large fluctuations in acid occurred without change in the total soluble solids and vice versa. Significant negative correlations were found to exist, however, between total soluble solids and total acids.

Due to the relatively high buffer capacity of the orange juice, large fluctuations in total acidity occurred without change in pH; but over a wide range of acid concentration (0.70 to 2.60 per cent.), the pH increased with a decrease in total acidity.

The experimental results show that the buffer capacity of orange juice is due chiefly to organic acids and inorganic salts.

The soluble pectins in the juice affected its buffer curve only slightly.

Boiling for 45 minutes, increased the initial pH of orange juice 0.2 over that of normal juice. This resulted in raising the buffer curve to a slightly higher pH level, but the shapes of the curves were the same.

Although the limits of dilution were not reached in these experiments, diluted (1:4) and undiluted juice had the same buffer capacity.

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LITERATURE CITED

1. APPLEMAN, DAVID, and RICHARDS, ANDREW V. Variability of sugar-acid ratio and total nitrogen in Valencia oranges. Proc. Amer. Soc. Hort. Sci. **37**: 539–542. 1939.
2. ASKEW, H. O. pH values and titratable acidity of apple-juices. New Zealand Jour. Sci. and Technol. **18**: 131–136. 1936.
3. BAIER, WILLARD E., and HIGBY, RALPH H. Measurement of ratio and color of citrus fruits. California Citrog. **16**: 202, 260. 1931.
4. BARNES, ELEANOR OLDS. Biochemical studies of some varieties of apples, plums, and grapes grown in Minnesota. Minnesota Agr. Exp. Sta. Tech. Bull. 143. 1940.
5. BARTHOLOMEW, E. T., and SINCLAIR, WALTON B. Unequal distribution of soluble solids in the pulp of citrus fruits. Plant Physiol. **16**: 293–312. 1941.
6. BLISH, M. J. Report on diastatic value of flour. Jour. Assoc. Off. Agr. Chem. **16**: 497–504. 1933.
7. ————. Report on diastatic value of flour. Jour. Assoc. Off. Agr. Chem. **17**: 394–399. 1934.
8. CALDWELL, JOSEPH S. Hydrion concentration changes in relation to growth and ripening in fruits. U. S. Dept. Agr. Tech. Bull. 403. 1934.
9. CHACE, E. M., and CHURCH, C. G. Maturity data on the California Washington Navel orange. California Citrog. **15**: 534, 569. 1930.
10. DENNY, F. E. Formulas for calculating number of fruits required for adequate sample for analysis. Bot. Gaz. **73**: 44–57. 1922.
11. DUNNE, T. C. Plant buffer systems in relation to the absorption of bases by plants. Hilgardia **7**: 207–234. 1932.
12. HAGEDORN, H. C., and JENSEN, B. N. Zur Mikrobestimmung des Blutzuckers mittelst Ferricyanid. Biochem. Zeitschr. **135**: 46–58. 1923.
13. ————, and ————. Die Ferricyanidmethode zur Blutzuckerbestimmung. Pt. 2. Biochem. Zeitschr. **137**: 92–95. 1923.
14. HILGEMAN, R. H., and SMITH, J. G. Changes in invert sugar and sucrose during ripening of Arizona grapefruit. Proc. Amer. Soc. Hort. Sci. **37**: 535–538. 1939.
15. HODGSON, R. W., and EGGERS, E. R. Rootstock influence on the composition of citrus fruits. California Citrog. **23**: 499, 531. 1938.

16. MARSH, GEORGE L.   Buffering action of nonacid vegetables.   Hilgardia
    11 : 315–341.   1938.

17. SINCLAIR, WALTON B., and BARTHOLOMEW, E. T.   Effect of rootstock on
    organic and inorganic constituents of citrus fruits.   Hilgardia.
    (In press.)

18. SNEDECOR, GEORGE W.   Statistical Methods.   3rd ed.   Collegiate Press,
    Inc., Ames, Iowa.   1940.

19. STEVENS, J. W., and BAIER, W. E.   Refractometric determination of
    soluble solids in citrus juices.   Ind. & Eng. Chem. Anal. Ed. 11 :
    447–449.   1939.

20. WEBBER, H. J.   Variations in citrus seedlings and their relation to
    rootstock selection.   Hilgardia 7 : 1–79.   1932.

MOLECULAR EQUIVALENCE OF CARBOHYDRATES TO CARBON DIOXIDE IN PHOTOSYNTHESIS

JAMES H. C. SMITH

Introduction

The photosynthetic reaction is commonly summarized by the following equation:

$$6\ CO_2 + 6\ H_2O = C_6H_{12}O_6 + 6\ O_2$$
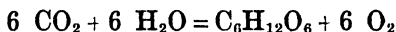
The organic material formed has been commonly assumed to be glucose or some other carbohydrate. This equation is based principally on three types of facts: (a), the preponderance of carbohydrate material in plants, and the observed rapid increase in carbohydrates on illumination of green plants; (b), the near quantitative agreement of the increase in weight of organic matter produced and the amount of carbon dioxide photosynthesized; and (c), the approximation to the value of unity of the ratio between oxygen evolved and carbon dioxide absorbed.

The first of these facts, (a), is only qualitative and does not serve to establish the stoichiometric relations required by the equation. The investigations thus far reported, that have attempted to confirm the molecular equivalence between carbon dioxide assimilated and carbohydrate formed, have failed in their purpose as a brief review will show.

Fifty years ago, SAPOSCHNIKOFF (12) determined, as glucose, the amount of carbohydrate formed during photosynthesis in relation to the amount of carbon dioxide absorbed. In three experiments with sunflower leaves, he found that 63.8, 67.7 and 87.1 per cent. of the theoretical amount of carbohydrate was formed from the carbon dioxide absorbed. Because he found so great a discrepancy between the observed and theoretical recovery of carbohydrate, he concluded that "Bei allen drei Versuchen erhielt ich ein Deficit der Kohlenhydrate im Vergleich zu der zersetzten Kohlensäure. Auf diese Versuche hin muss man annehmen, dass ausser den Kohlenhydraten (Stärke) sich noch ein anderer Stoff bildet, und vielleicht ist dies Eiweissstoff."

Later KRASCHENINNIKOV (8, 14b) examined five different plants and found the following ratios: (A), the increase in dry weight as compared to the carbon dioxide absorbed; (B), the increase in carbohydrate in relation to the carbon dioxide absorbed; and (C), the increase in carbohydrate as related to the increase in dry weight.

	A	B	C
Bamboo	0.60	0.45	0.75
Cherry laurel	0.60	0.31	0.51
Sugar cane	0.67	0.50	0.75
Linden	0.75	0.56	0.75
Tobacco	0.65	0.37	0.57

From these results it can be seen that the average ratio of increase in dry weight to carbon dioxide absorbed, 0.654, closely approximates the theoretical value for a disaccharide, 0.648. This supports the supposition that the photosynthate is carbohydrate. Such close agreement may be misleading, however, for it may result from the accumulation of several products the sum of whose weights may equal fortuitously the weight of the hypothetical carbohydrate. The fact that the average increase in carbohydrates, reckoned as glucose, is only 64.2 per cent. of the amount required by theory, may be used to uphold the latter interpretation.

The ratio between the increase in dry weight of leaves of *Catalpa bignonioides* and the amount of carbon dioxide assimilated, was found by BROWN and ESCOMBE (3), using the half-leaf method of analysis, to be much larger than the increase expected on the assumption that the material synthesized was carbohydrate. This difference was ascribed to errors inherent in the method of determination.

In the most recent experiments, in which carbon isotopes have been used to estimate the amount of material formed, the results indicate that carbohydrate is not the only class of substance formed by photosynthetic action. The experiments carried out by RUBEN, HASSID, and KAMEN (11) on barley plants, using radioactive carbon, C^{11}, showed that only 25 per cent. of the carbon fixed by photosynthesis was water-soluble carbohydrate, and that not more than 10 per cent. could be contained in the insoluble material. From these experiments it was concluded that "The bulk of the radioactive material found in the plant is water soluble and is not carbohydrate. . . ." Recently heavy carbon, C^{13}, has been used for the investigation of the carbon metabolism of bean and radish plants. In an abstract of a paper given by BELKENGREN, NIER, and BURR (2), it is stated that "The conversion of newly formed photosynthate into chlorophyll, xanthophyll, lipids, cellulose, starch, protein, amino acids and amides has been measured." Whether or not the carbon first incorporated into the plant is in the form of diffusible carbohydrate was not stated.

The third type of observation that has led to the acceptance of the usual photosynthesis equation is the close approach to unity of the photosynthetic quotient, *i.e.*, the ratio of oxygen evolved to carbon dioxide absorbed. SPOEHR (15) has recently pointed out that even though the photosynthetic quotient were always equal to 1, this is no proof that the organic products formed consist solely of carbohydrates. So small a variation from unity as 3 per cent. as was found by MAQUENNE and DEMOUSSY (10), might indicate the formation of as much as 12 per cent. of protein. The fact that this ratio has been found in many cases to differ from unity by a considerable amount (14a, 17), and the fact that the quotient has been found to vary during the time course of photosynthesis (6, 8), also suggest that the process may not always conform to the equation cited, and that organic products other than carbohydrates may be synthesized during illumination.

From this short summary it is obvious that the facts are insufficient to

establish the widely accepted concept of the photosynthetic reaction, particularly the concept that carbohydrates are the exclusive organic products of photosynthesis. In order to gain more precise knowledge concerning the substances produced by the photosynthetic process, the quantitative relation between the amount of carbon dioxide absorbed and the nature and quantity of organic matter formed during photosynthesis should be investigated rigorously for a number of species of plants. As a contribution to this subject we have investigated this relation for sunflower leaves.

Experimentation

In these experiments the increase in carbon content of sunflower leaves, brought about by photosynthesis, was determined and compared with the corresponding increase in dry weight. The increase in carbon content was obtained by measuring the uptake of carbon dioxide during photosynthesis, and also by determining the gain in carbon by elementary analysis. Furthermore, the increase in various carbohydrate constituents of sunflower leaves was determined and related to the amount of carbon dioxide absorbed during illumination of the leaves.

METHOD OF MEASURING CARBON DIOXIDE ABSORPTION

The amount of carbon dioxide taken up by the sunflower leaves was measured by determining the decrease in carbon dioxide concentration in a closed system. The arrangement was such that gas was circulated through the component parts of the apparatus in the following order: leaf chamber, gas pipet, pump, pH-measuring cell, and back to the leaf chamber again. The leaf chamber employed has already been described by SPOEHR (14c). An "Autopulse Fuel Pump" reconstructed so as to be gas-tight, was used to circulate the gas within the system. The pH-measuring cell used in a previous investigation (13) was employed in this investigation. The cell contained a solution, 0.01 N with respect to sodium bicarbonate, and 1.0 N with respect to potassium chloride. The decrease in carbon dioxide concentration was determined from the change in pH of this solution, which was in equilibrium with the carbon dioxide in the circulating gas stream. A "Beckman pH Meter" was used to measure the pH of the bicarbonate solution. It was possible to read the pH accurately to 0.01 pH unit. The gas pipet, at 20° C., contained 28.604 mg. of carbon dioxide. It was fitted with two three-way stopcocks so that it could be cut out of the circulation system to be flushed and filled with pure carbon dioxide; then when desired, it could be cut into the circulation system so as to introduce a known volume of carbon dioxide. A by-pass around the gas pipet permitted circulation of the gas within the system without the gas passing through the pipet. Carbon dioxide for filling the pipet was taken from a commercial cylinder. The whole apparatus except the pump and some of the glass tubing, was immersed in a constant temperature bath, controlled either at 20.0° C. ± 0.1 or 10.0° ± 0.1. The walls of the water bath contained windows so that the leaf could be illuminated from the side.

After the leaf had been introduced into the leaf chamber, the apparatus was closed and the gas circulated within the system until the rate of respiration had become constant. The measured quantity of carbon dioxide was then introduced from the gas pipet. When equilibrium between the gas and the bicarbonate solution had been established (about 15 minutes was required for this), the leaf was illuminated by an approximately parallel beam from a 500-watt projection lamp. Removal of carbon dioxide by photosynthesis caused the pH to increase progressively. When approximately all of the added carbon dioxide had been used up, the light was turned off and the pH readings taken until a maximum value was reached, at which time the experiment was terminated. The amount of carbon dioxide absorbed by the leaf was calculated from the volume of the pipet, and the pH readings taken at the following times: when the carbon dioxide was introduced; when the light was turned on; and when the experiment was stopped.

Increased accuracy of measurement was obtained by arranging the experiment so that nearly the same quantity of carbon dioxide was absorbed as was pipetted into the apparatus. Under such conditions, the pH values obtained before introducing the carbon dioxide and after terminating the illumination were about equal. At this relatively high pH the small deviations in pH represented very small differences in carbon dioxide concentration. These differences were only a small fraction of the accurately measured quantity of carbon dioxide introduced, and any error introduced on account of inaccuracies in measuring them was entirely negligible. This technique made the method essentially a null-point method.

METHODS OF ANALYSIS

DETERMINATION OF THE RATIO OF THE INCREASE IN DRY WEIGHT TO THE AMOUNT OF CARBON DIOXIDE ABSORBED.—In each of these experiments, the leaf which was used was taken from a plant which had been in the dark at least overnight. The petiole was cut from the stalk of the plant and immediately plunged into water. After rinsing the leaf with distilled water, the surface of the leaf was freed of adhering water. The halves of the leaf were then cut from the midrib and the areas outlined on sheets of heavy cellophane. The areas were measured by means of a planimeter. One half of the leaf was placed in a weighing bottle and dried at 85° to 90° C., first in a stream of air, and then in an ordinary drying oven. The other half of the leaf was fastened in a wire frame so as to hold the leaf flat and then placed in the leaf chamber of the photosynthesis apparatus.

The respiration of the leaf in the photosynthesis apparatus was measured so that proper correction could be made for the carbon lost by respiration. The correction had to be estimated for the period between the starting of the drying of the control sample and the beginning of the illumination of the photosynthesis sample. The correction usually amounted to about 2 or 3 per cent. of the amount of carbon dioxide absorbed.

When the measurement of the respiration was completed carbon dioxide was introduced into the photosynthesis apparatus, the light turned on, and photosynthesis carried out for the desired period. After the uptake of carbon dioxide had been measured (table I, col. 2), the leaf half was transferred to a weighing bottle and the dry weight determined exactly as described for the control.

The gain in carbon was also determined from elementary carbon analysis. The percentages of carbon in the dried material, from both the control sample (table I, col. 7) and photosynthesis sample (table I, col. 8) were obtained by the method of "Manometric Carbon Determination" described by VAN SLYKE and FOLCH (18). The weight of carbon in the control sample, corrected to a sample with an area equal to that of the photosynthesis

TABLE I

INCREASE IN DRY WEIGHT COMPARED WITH INCREASE IN CARBON ABSORBED
DURING PHOTOSYNTHESIS

EXPERIMENT NO.	CARBON ABSORBED AS CO_2	INCREASE IN CARBON BY COMBUSTION	INCREASE IN DRY WEIGHT	$\triangle$ CARBON ABSORBED/$\triangle$ DRY WT.	$\triangle$ CARBON BY COMBUSTION/$\triangle$ DRY WT.	PERCENTAGE OF CARBON IN DRY WEIGHT OF SAMPLE	
						CONTROL	PHOTOSYNTHESIS
	mg.	*mg.*	*mg.*	%	%	%	%
1	5.278	5.223	12.9	40.9	40.5	46.00	45.58
2	7.560	8.907	20.6	36.7	43.2	44.75	44.98
3	7.302	8.037	17.2	42.5	46.7	45.30	45.48
4	6.783	6.466	17.6	38.5	36.7	47.00	45.50
5	6.025	6.222	14.1	42.7	44.1	46.12	45.90
6	6.186	5.554	14.0	44.2	39.7	45.25	44.67
	Average			40.9	41.8	45.74	45.35
	Probable error			± 0.9	± 1.1	± 0.24	± 0.18

sample, was subtracted from the amount of carbon in the photosynthesis sample. The difference was ascribed to carbon gained by photosynthesis. The values obtained in the various experiments are shown in table I, column 3. The wet combustion of leaf material had distinct advantage because the carbon in carbonates as well as organic carbon was determined quantitatively. This particular method was especially useful in the present investigation because of its speed and high degree of accuracy.

The gain in dry weight was obtained by subtracting the dry weight of the control sample, adjusted to an initial area equal to that of the photosynthesis sample, from the dry weight of the photosynthesis sample. The increases in dry weight obtained in different experiments are given in table I, column 4. The ratios of the gain in carbon, obtained from carbon dioxide absorption and from elementary carbon analysis, to gain in dry weight, are shown in table I, columns 5 and 6. It is evident that the ratios obtained by the two methods agree very well.

DETERMINATION OF THE RATIO OF THE INCREASE IN CARBOHYDRATES TO THE AMOUNT OF CARBON DIOXIDE ABSORBED.—In order to determine the increase in carbohydrates in relation to the amount of carbon dioxide absorbed, the following procedure was employed. The leaf used in each of the experiments was cut in the afternoon from a plant in the greenhouse. The petiole was plunged immediately into water. After a few minutes the leaf was washed with distilled water and kept overnight in a dark cabinet, the petiole being immersed in distilled water. (This technique is in contradistinction to the technique described in the preceding section in which the whole plant had been kept in darkness and the leaf was used for experimentation very shortly after being cut. The extended dark treatment in both cases was for the purpose of depleting the carbohydrate content of the leaf.) In the morning the halves of the leaf were cut from the midrib and each half cut in two transversally. The two diagonal quarters served for the control sample and the two remaining quarters for the photosynthesis sample. The leaf samples were placed in covered weighing bottles and weighed. The two quarters that were to be used for the control were spread out in the dark cabinet, with upper side down. At the time when illumination of the photosynthesis sample was begun, the control sample was removed from the dark cabinet and dropped into boiling 80 per cent. alcohol. By this procedure an equal period of respiration, prior to illumination, was secured for both control and photosynthesis samples.

The two quarters to be used for photosynthesis were fastened in a wire frame and brought into the photosynthesis apparatus. After respiration had become constant, the carbon dioxide was pipetted into the circulating gas stream. When equilibrium with the increased concentration of carbon dioxide had been established the light was turned on. Photosynthesis was allowed to continue until the carbon dioxide taken up by leaf approximately equaled the amount introduced, then the light was turned off. When the pH reached a maximum (in about 10 minutes at 20°, and 15 to 20 minutes at 10°), the experiment was stopped. The leaf was removed from the apparatus and immersed immediately in boiling 80 per cent. alcohol.
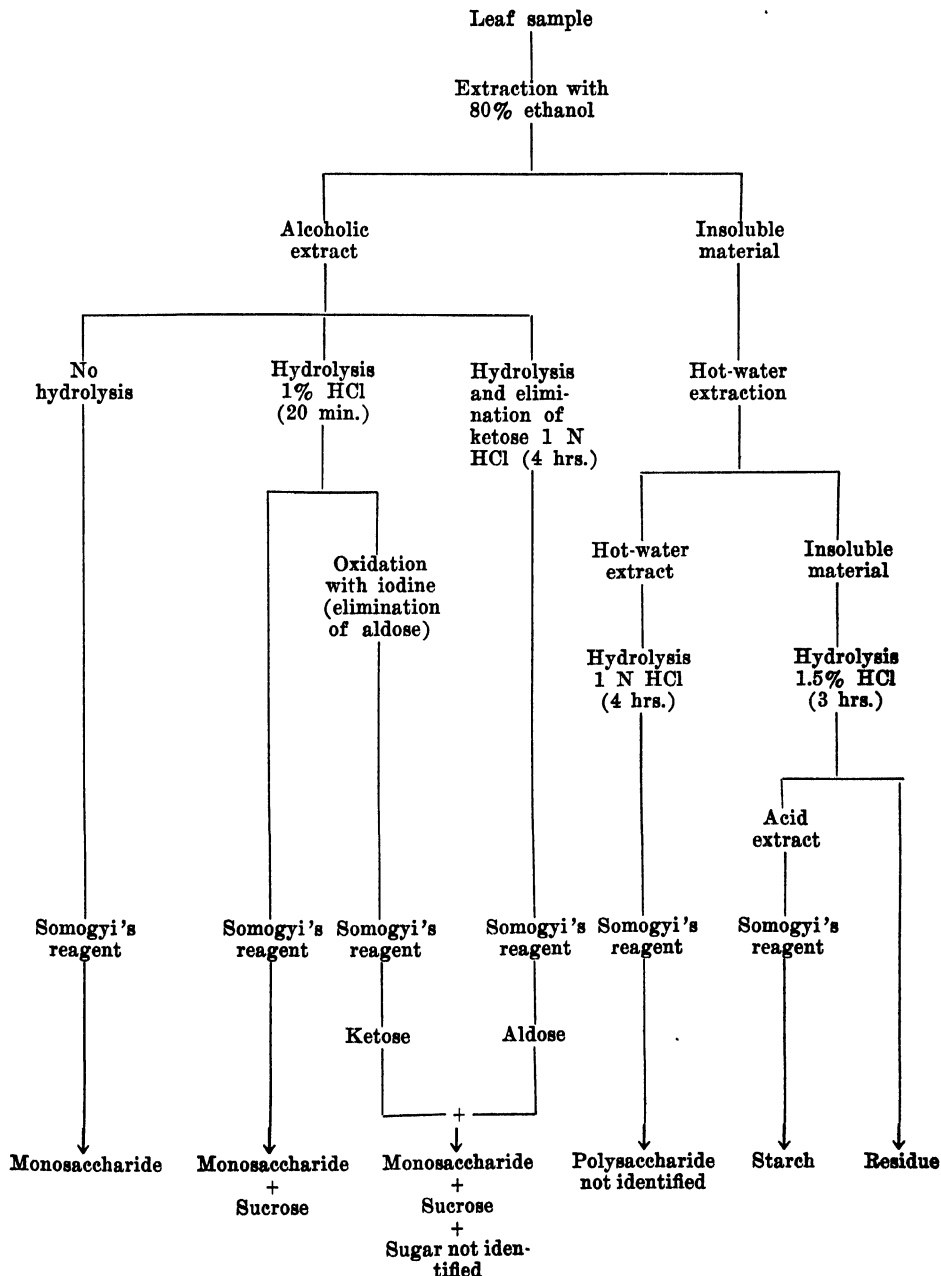
In some experiments at 20° an attempt was made to determine the fate of the new-formed carbohydrates immediately following their synthesis. For this purpose a prolonged period of respiration was introduced immediately following the short period of photosynthesis. At the close of the experiment, increases in the various fractions of carbohydrates and in the residue were determined and were correlated with the net gain in carbon absorbed as carbon dioxide. The term net gain is used to designate the difference between the total amount of carbon dioxide absorbed during photosynthesis minus the amount of carbon dioxide lost during the prolonged period of respiration.

In the photosynthesis experiments carried out at 10°, the control sample was placed in a refrigerator at about 5° for the period between weighing and immersion in the alcohol.

A synopsis of the procedure for carbohydrate analysis is given in the

form of a flow sheet. The various carbohydrates determined appear at the bottom of the diagram, and these are designated by the same terms that are used in the succeeding tables.

FLOW SHEET OF CARBOHYDRATE ANALYSIS

Leaf sample

Extraction with
80% ethanol

Alcoholic
extract — Insoluble
material

No
hydrolysis

Hydrolysis
1% HCl
(20 min.)

Hydrolysis
and elimi-
nation of
ketose 1 N
HCl (4 hrs.)

Hot-water
extraction

Oxidation
with iodine
(elimination
of aldose)

Hot-water
extract — Insoluble
material

Hydrolysis
1 N HCl
(4 hrs.)

Hydrolysis
1.5% HCl
(3 hrs.)

Acid
extract

Somogyi's
reagent

Somogyi's
reagent

Somogyi's
reagent

Somogyi's
reagent

Somogyi's
reagent

Somogyi's
reagent

Ketose   Aldose

Monosaccharide

Monosaccharide
+
Sucrose

Monosaccharide
+
Sucrose
+
Sugar not iden-
tified

Polysaccharide
not identified

Starch   Residue

EXTRACTION.—After immersion in the alcohol the two samples were treated by as nearly identical procedures as possible. The leaf material was extracted five times with 20-ml. portions of boiling 80 per cent. ethanol. The alcoholic extracts after filtration were collected and analyzed for the dissolved sugars. The leaf material was then extracted four times with 20 ml. each time, of hot distilled water. The aqueous extracts were filtered, collected, and analyzed for the carbohydrates contained therein. The leaf material remaining from the hot-water extraction was suspended in about 20 ml. of 1.5 per cent. hydrochloric acid and hydrolyzed in a boiling water bath for 3 hours. The hydrolyzate was filtered into a 100-ml. volumetric flask. The leaf residue was extracted with hot water, three times with 20 ml. each, and once with 15 ml. These extracts were filtered into the same volumetric flask. The solution was diluted to volume and analyzed for sugar. Each extraction was carried out on the boiling water bath for a period of 20 minutes. The leaf material which had remained insoluble throughout all these extractions was collected in the same filter paper that had been used to filter each of the extracts, and was quantitatively transferred to a weighing bottle and the dry weight determined.

Analysis

ALCOHOLIC EXTRACT

The alcoholic extract was evaporated on the water bath to a small volume. Water (20 ml.) was added and the solution evaporated until the odor of alcohol had disappeared. The concentrated solution was transferred quantitatively to a centrifuge tube, 1 ml. of lead acetate solution (about 14 per cent.) added, and after a few minutes the precipitate was thrown down by centrifugation. The supernatant liquid was decanted into a 100-ml. volumetric flask containing 2 ml. of saturated sodium oxalate solution. The residue was washed three times by centrifugation with distilled water and the washings collected in the volumetric flask. After all of the lead oxalate had precipitated the solution was made to volume, thoroughly mixed, and the precipitate removed by filtration. In the filtrate, monosaccharides, sucrose, and a "sugar not identified" were determined by means of SOMOGYI's method (1). A reduction period of 25 minutes was employed (7).

The monosaccharides were estimated by determining the reducing power of the sugar solution without further treatment. Sucrose was determined by obtaining the increase in reducing sugar after a 20-minute hydrolysis with 1 per cent. hydrochloric acid. The hydrochloric acid was neutralized with sodium hydroxide using rosolic acid indicator and the sugars determined by reduction. The "sugar not identified" was determined by estimating the increase in reducing power brought about by hydrolysis with 1 N hydrochloric acid for 4 hours. The details of the method were as follows:

The sugar solution (5.00 ml.) was pipetted into a Pyrex test tube, 25 × 250 mm., and 0.46 ml. of concentrated hydrochloric acid added. The solution was hydrolyzed for 4 hours in the boiling water bath. After neu-

tralization of the acid with sodium hydroxide, the reducing power was determined.

To a duplicate sample, after hydrolysis, just enough solid sodium bicarbonate was added to neutralize the acid, the carbon dioxide evolved was removed by evacuation at the water pump, then 0.5 ml. of a buffer solution composed of potassium bicarbonate and potassium carbonate (ca. 2.5 N with respect to potassium ions, pH = 9.60) was introduced. This brought the solution to a pH of ca. 9.25, an optimal value for oxidizing glucose without appreciably attacking levulose. Iodine solution (0.3 ml. of 0.2 N) was in-

TABLE II

TABULAR PRESENTATION OF A SAMPLE EXPERIMENT. TEMPERATURE: 20.0° C.*

FRACTIONS	CONTROL	CONTROL CORR. FOR INITIAL WEIGHTS	PHOTO-SYN-THESIS	INCREASE IN CARBO-HYDRATE	INCREASE IN CARBON	RECOVERY OF CARBON ABSORBED
	mg.	mg.	mg.	mg.	mg.	%
Initial fresh weight of sample	2661.7		2691.2			
Carbon absorbed					7.871	
Monosaccharide	7.740	7.826	10.760	2.934	1.174	14.9
Sucrose	4.720	4.772	15.280	10.508	4.203	53.4
Sugar not identified	1.784	1.804	2.564	0.760	0.304	3.9
Polysaccharide not identified	9.780	9.889	10.550	0.661	0.264	3.4
Starch	17.840	18.038	22.640	4.602	1.841	23.4
Total soluble carbohydrate					7.786	98.9
Residue	70.9	71.7	74.5	2.80	1.244	15.8
Total recovery	-				9.030	114.7

* Explanation of table II. Col. 1: Designation of different fractions. Cols. 2 and 4: Weights of various fractions for the control, col. 2, and photosynthesis sample, col. 4; weights of carbohydrate fractions in terms of glucose, and actual weights of samples and residues. Col. 3: The values in col. 2 multiplied by the ratio of the initial fresh weights of photosynthesis sample to control sample. Col. 5: Values in col. 4 minus corresponding values in col. 3. Col. 6: The amount of carbon absorbed. Increase of carbon in each fraction: values for carbohydrates multiplied by 0.4000, the carbon content of glucose; value for residue multiplied by 0.4444, the carbon content of cellulose. Col. 7: The percentage of the carbon absorbed recovered in each fraction.

troduced and the mixture allowed to stand for 60 minutes. After acidification with 0.3 ml. of 6 N hydrochloric acid, the liberated iodine was reduced with 2 per cent. sodium sulphite solution. The solution was neutralized with 6 N sodium hydroxide (rosolic acid indicator being used), 0.5 ml. of a glucose solution of known titre was added, and the reducing power of the solution was determined. By deducting the amount of added glucose, and estimate of the ketoses, presumably levulose, left undestroyed by the prolonged hydrolysis, was obtained. Subtraction of the amount of undestroyed levulose from the total amount of monosaccharide contained in the hydrolysis mixture provided an estimate of the total quantity of aldoses, herein

TABLE III

AMOUNT OF CARBOHYDRATES FORMED RELATIVE TO THE AMOUNT OF CARBON ABSORBED. TEMPERATURE: 20.0° C.*

EXPERIMENT NO. →	1	2	3	4	5	6	7	RECOVERY OF CARBON ABSORBED
MINUTES ILLUMINATION →	59	47	60	83	50	64	42	%
INCREASE IN CARBON								
	mg.	mg.	mg.	mg.	mg.	mg.	mg.	
Carbon absorbed	7.768	7.871	7.736	7.822	7.832	7.477	7.895	
Monosaccharides	0.260	1.174	1.263	0.718	0.860	0.826	0.326	10.0
Sucrose	4.110	4.203	3.966	4.839	4.591	2.926	3.609	51.8
Sugar not identified	0.389	0.304	0.256	0.168	0.617	0.000	-0.036	3.1
Polysaccharide not identified	-0.034	0.264	0.024	-0.174	-0.079	0.029	0.719	1.4
Starch	2.166	1.841	2.007	1.030	1.493	2.884	2.446	25.5
Total soluble carbohydrate	6.891	7.786	7.516	6.581	7.482	6.665	7.064	
Recovered as soluble carbohydrate: % of C absorbed	88.7	98.9	97.2	84.1	95.5	89.1	89.5	91.9 ± 1.5
Residue†	0.311	1.244	0.444	-0.444	0.711	0.800	0.471	6.5
Total carbon recovered	7.202	9.030	7.960	6.137	8.193	7.465	7.535	
Total recovery: % of C absorbed	92.7	114.7	102.9	78.5	104.6	99.8	95.4	98.4 ± 3.1

* Explanation of table III. Col. 1: Designation of the different fractions determined. Cols. 2 to 8: The increase of carbon in the different fractions. The values in these columns correspond to the values recorded in col. 6, table II. Col. 10: The percentage of the total carbon absorbed that was recovered in the different fractions.

† Carbon calculated from the increase in weight of the residue by multiplying by 0.4444, the percentage of carbon in cellulose.

designated glucose. The vigorous hydrolysis destroyed part of the glucose.
By separate experiment this was found to be 4 per cent. [*Cf.* DAVIS and
DAISH (5).] To obtain the true value for glucose the observed value was
increased by 4 per cent.

In order to determine the total amount of levulose, the solution was
heated in the boiling water bath for 20 minutes with 1 per cent, hydrochloric
acid, and the reducing power determined after oxidation with iodine in the
manner already described. The amount of levulose obtained by this analysis
added to the total amount of glucose obtained by vigorous hydrolysis, yielded

TABLE IV

AMOUNT OF CARBOHYDRATES FORMED RELATIVE TO THE AMOUNT OF CARBON ABSORBED.
TEMPERATURE: 10.0° C.*

EXPERIMENT NO. →	1	2	3	4	RECOVERY OF CARBON ABSORBED
MINUTES ILLUMINATION →	153	145	159	148	
	INCREASE IN CARBON				
	mg.	*mg.*	*mg.*	*mg.*	%
Carbon absorbed	8.050	8.081	8.064	8.073	
Monosaccharides	0.349	1.080	0.663	0.229	7.1
Sucrose	6.788	4.648	5.638	5.819	71.0
Sugar not identified	0.318	0.736	0.260	0.289	5.0
Polysaccharide not identified	− 0.073	0.139	− 0.521	0.292	− 0.5
Starch	0.673	1.941	1.610	0.960	16.1
Total soluble carbohydrate	8.055	8.544	7.650	7.589	
Recovered as soluble carbohydrate: % of C absorbed	100.1	105.7	94.9	94.0	98.7 ± 2.1
Residue†	1.313	− 0.298	1.848	− 0.316	7.9
Total carbon recovered	9.368	8.246	9.498	7.273	
Total recovery: % of C absorbed	116.4	102.0	117.8	90.1	106.6 ± 5.0

* *Cf.* explanation of table III; see footnote (*) table III.
† From elementary carbon analyses.

the total amount of reducing sugars obtainable from this solution. From
this total quantity was subtracted the amount of sugars obtained by mild
hydrolysis, *viz.*, glucose and sucrose. The difference was designated, "sugar
not identified."

Separate experiments demonstrated that analyses of mixtures of glucose
and sucrose made by both the mild hydrolysis method and the vigorous
hydrolysis method supplemented with the iodine oxidation, agreed on the
average within 1.5 per cent.[1]

In summary, the sugars determined by the procedures described in this
section were: monosaccharides, sucrose, and "sugar not identified."

[1] For a discussion of this type of methods for the determination of ketoses, *cf.*
BROWNE and ZERBAN (4).

HOT-WATER EXTRACT

The solution obtained by extraction of the leaf material with hot water
was concentrated to approximately 10 ml. on the boiling water bath. The
concentrate was transferred quantitatively to a 50-ml. volumetric flask and
diluted to the mark. After hydrolysis for 4 hours at 100° C. with 1 N
hydrochloric acid, the reducing power was determined. The carbohydrate
estimated in this way was designated "polysaccharide not identified."

ACID EXTRACT

The solution prepared by hydrolysis of the polysaccharides with 1.5 per
cent. hydrochloric acid and extraction of the soluble sugars thus formed
was neutralized and the reducing power of the solution determined. The
carbohydrate determined in this manner was called starch.

In all determinations the analytical data for the control sample were
adjusted to correspond to a sample with the initial weight of the photo-
synthesis sample. After making this adjustment, the difference in weights
found in the corresponding categories of carbohydrates for the photosyn-
thesis and the control samples was taken to be the increase in weight caused
by photosynthesis. A typical experiment is detailed in table II. A summary
of the increases of carbon in the carbohydrate and residue fractions corre-
lated with the amount of carbon absorbed is given in tables III to V.

TABLE V

CORRELATION OF CARBOHYDRATES RECOVERED WITH THE INCREASE IN CARBON.
PHOTOSYNTHESIS FOLLOWED BY PROLONGED RESPIRATION.
TEMPERATURE: 20.0° C.*

EXPERIMENT NO. →	1	2	
MINUTES ILLUMINATION →	84	57	RECOVERY OF CARBON: NET GAIN
MINUTES RESPIRATION AFTER ILLUMINATION →	196	243	
	INCREASE IN CARBON		
	mg.	mg.	%
Carbon absorbed: total	7.538	8.063	
Carbon absorbed: net gain	6.331	6.425	
Monosaccharides	2.202	2.249	34.9
Sucrose	2.766	2.964	44.9
Sugar not identified	0.091	0.564	5.1
Polysaccharide not identified	− 0.026	− 0.176	− 1.6
Starch	1.149	1.153	18.1
Total soluble carbohydrate	6.182	6.754	
Recovered as soluble carbohydrate: % of net gain in carbon	97.6	105.1	101.4
Residue†	0.044	− 0.088	− 0.3
Total carbon recovered	6.226	6.666	
Total recovery: % of net gain in carbon	98.3	103.8	101.1

* For explanation of the significance of the columns, cf. table III, footnote (*).
† Cf. table III, footnote (†).

There was indication that pentoses or pentosans were present in the hot-water and the acid extracts. Even though these carbohydrates may have increased to a slight extent during photosynthesis, the percentage recovery of carbon in carbohydrates would have been little influenced by their increase because of the similarity in reducing power of the pentoses and glucose.

Discussion

From the ratio of the increase in carbon to the increase in dry weight, the percentage of carbon in the photosynthate was determined. From six experiments (table I) the percentage of carbon in the photosynthate was found to be 40.9 ± 0.9 per cent. when calculated from the amount of carbon dioxide absorbed. When reckoned from combustion analysis, it was found to be 41.8 ± 1.1 per cent. The average, 41.4 ± 0.6 per cent., approximates the percentage of carbon in a disaccharide, 42.10 per cent. This is indirect evidence that the photosynthate is carbohydrate in nature.

It may be of interest to note that the carbon percentage of the dried leaf portion used as control was found to be 45.74 ± 0.24 per cent. This value, when a reasonable correction for ash is made, would be raised to 51 or 52 per cent., which is considerably higher than the carbon content of the photosynthate, 41.4 per cent., or even of cellulose, 44.44 per cent. The causes for the accumulation in the leaf of compounds of higher carbon content than that of the photosynthate are not known. Two explanations of the effect may be suggested: (a) The photosynthate may be transformed into compounds of higher carbon content by metabolic processes of the leaf such as dehydration, cyclization, and reduction. (b) The photosynthate, although preponderantly carbohydrate may contain small quantities of materials of higher carbon content. Through preferential respiration, the carbohydrates may be used up. This would permit accumulation of the compounds of higher carbon content.

The nature and quantity of the organic matter formed was also determined by direct analysis of the leaf constituents. As it turned out, most of the constituents, which increased on illumination of the leaf, could be converted into substances which reduced alkaline copper reagent (SOMOGYI's reagent) and so were determined by this reagent. The increase in reducing power brought about by photosynthesis was calculated as glucose. An increase in the weight of the residual material, which remained insoluble throughout all the procedures employed, was also observed. This was a small part of the total increase. As yet the nature of this portion of the photosynthate is not known. It may be carbohydrate, i.e., cellulose, hemicellulose, etc., or it may be protein inasmuch as the residue contained nitrogen.

In table VI are tabulated the increases observed in various classes of organic substances expressed as percentages of the carbon absorbed by the assimilation of a known amount of carbon dioxide. An examination of table VI shows that the recovery of carbon absorbed is very close to 100 per cent. Because so large a fraction of the assimilated carbon dioxide has been

TABLE VI

COMPARISON OF THE PROPORTIONS OF THE DIFFERENT CARBOHYDRATE FRACTIONS
OBTAINED AT DIFFERENT TEMPERATURES

NUMBER OF EXPERIMENTS INCLUDED →	4	7
TEMPERATURE →	10.0°	20.0°
AVERAGE TIME OF ILLUMINATION: MINUTES →	156	58
	RECOVERY OF CARBON GAINED	
	%*	%†
Monosaccharide	7.1	10.0
Sucrose	71.0	51.8
Sugar not identified	5.0	3.1
Polysaccharide not identified	− 0.5	1.4
Starch	16.1	25.5
Total soluble carbohydrate	98.7 ± 2.1	91.9 ± 1.5
Residue	7.9	6.5
Total recovery	106.6 ± 5.0	98.4 ± 3.1

* From table IV, col. 6.
† From table III, col. 9.

recovered as carbohydrate, especially at 10° C., it is apparent that the equivalence between carbon dioxide and carbohydrate required by the photosynthesis equation has in this instance been established.

While, on the average, the residues of the illuminated portions of the leaves showed an increase in weight, this was not always so. In fact, the variations were so great, especially at 10° C., as to cast doubt on the significance of the average increase observed.

Comparison of the results of the experiments carried out at 10° and 20° shows that at the lower temperature there was a greater recovery of carbon in sucrose and lesser recovery in starch and in monosaccharide. It is impossible to say at the present time whether this may be used as evidence to substantiate any of the alternative theories concerning the sugar first formed in photosynthesis.

If the assumption is made that carbohydrates are the sole products of photosynthesis (cf. table VI), then after a period of prolonged respiration the possibilities exist that the carbon recovered as carbohydrate will be less than, equal to, or greater than the net amount of carbon absorbed. If, during this period of respiration, the carbohydrates are transformed into other soluble substances the amount of carbohydrate recovered will be a smaller fraction of the net gain in carbon than if no respiration period had been allowed. If only carbohydrates are respired then the observed gain in carbohydrates should represent the same fraction of the net gain in carbon as when no respiration period was permitted. If, however, other organic substances are transformed into carbohydrates, or are respired in amounts comparable to the carbohydrates, then the possibility exists that the increase in carbohydrates will be greater with a long respiration period than without.

The results of the experiments were quite variable.   Approximations to all three of these conditions were found among the various experiments performed, although recovery in excess of 100 per cent. was never greater than the probable variation.   So far no generalization can be made concerning the types of reactions which occur in the absence of light.   Because of the variability observed in the different leaf samples it is conceivable that the type of reaction depends on the character or condition of the individual leaf used in the experiment.

A striking feature of the prolonged respiration was the change brought about in the relative proportions of the different carbohydrates.   For purposes of comparison, experiments employing long periods of respiration (table V) were chosen in which the total recovery of carbon was comparable to the recovery in the experiments with short respiration periods (table III). The comparison is shown in table VII.

TABLE VII

COMPARISON OF THE PROPORTIONS OF THE DIFFERENT CARBOHYDRATE FRACTIONS
OBTAINED IN EXPERIMENTS WITH AND WITHOUT A PROLONGED RESPIRATION
PERIOD FOLLOWING PHOTOSYNTHESIS.   TEMPERATURE: 20.0° C.

NUMBER OF EXPERIMENTS INCLUDED →	2	7
	min.	min.
AVERAGE TIME OF ILLUMI- NATION →	71	58
AVERAGE TIME OF ILLUMI- NATION + RESPIRATION →	290	66
	RECOVERY OF CARBON GAINED	
	%*	%†
Monosaccharides	34.9	10.0
Sucrose	44.9	51.8
Sugar not identified	5.1	3.1
Polysaccharide not identified	− 1.6	1.4
Starch	18.1	25.5
Total soluble carbohydrate	101.4	91.9
Residue	− 0.3	6.5
Total recovery	101.1	98.4

* From table V, col. 4.
† From table III, col. 9.

From this comparison it is apparent that over the period of respiration the monosaccharides have increased to a very great degree.   Starch and sucrose have both decreased.   Possibly there is a significant decrease in the material contained in the insoluble residue, although this is doubtful in view of the large variations observed.   It should be pointed out that under the conditions employed the leaves lost considerable water during photosynthesis and possessed a severe water deficit throughout the period of respiration. This may have influenced the change in the ratios of the different carbohydrates during the prolonged respiration period for it is well known that water deficit in sunflower leaves influences the dissolution of starch (16).

MECHANISM OF CARBOHYDRATE FORMATION

From the results set forth in this paper it is evident that the ratio of the amount of carbohydrate formed to the amount of carbon dioxide absorbed is very close to the equivalence demanded by the commonly accepted photosynthesis equation.   This close statistical correspondence, however, does not insure that the carbon atoms absorbed as carbon dioxide are the ones transformed into the carbohydrate molecules recovered.   If comparison of different species of plants is justifiable, a correlation of the facts observed in this investigation with the facts already obtained by the use of radioactive carbon (11) might indicate that a direct transformation of carbon dioxide into carbohydrate is not accomplished by the photosynthetic process, otherwise a greater recovery of radioactive carbohydrates would have been obtained in the experiments with labeled carbon.   To determine whether there is a direct conversion will require careful quantitative determinations to be carried out with labeled carbon on a number of different species of plants.

Conclusions and summary

From the results found in this investigation it may be stated that the equivalence demanded by the photosynthesis equation for the formation of carbohydrates from carbon dioxide has been demonstrated to be valid for sunflower leaves.   There is a temperature effect on the proportion of the different classes of carbohydrates recovered after short periods of photosynthesis.   This effect apparently favors the accumulation of disaccharides, such as sucrose, at the lower temperature.

The carbon content of the material accumulated during photosynthesis approaches that of a disaccharide.   It is considerably lower than the average carbon content of the organic material of the leaf.   This indicates that metabolic reactions subsequent to photosynthesis tend to convert the material photosynthesized into compounds of higher carbon content.

Respiration and transformation of the organic material formed during photosynthesis possibly follows different courses in different samples of sunflower leaves.   In most cases, however, the principal material respired seems to be carbohydrate.

The results set forth in this paper have been obtained from the examination of sunflower leaves alone.   Only after examination of a large number of species of plants will it be permissible to generalize more extensively.

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LITERATURE CITED

1. Assoc. Offic. Agr. Chemists. Official and tentative methods of analysis. Fifth edition. Washington, D. C., p. 506. 1940.

2. BELKENGREN, R., NIER, A. O., and BURR, G. O. Abstract of paper: Heavy carbon isotope in plant metabolism. Nature **149**: 24. 1942.

3. BROWN, H. T., and ESCOMBE, F. Researches on some of the physiological processes of green leaves, with special reference to the interchange of energy between the leaf and its surroundings. Proc. Royal Soc. (London). **B76**: 29–111. 1905.

4. BROWNE, C. F., and ZERBAN, F. W. Sugar analysis. John Wiley and Sons, New York, Third Edition. pp. 902–904. 1941.

5. DAVIS, W. A., and DAISH, A. J. A study of the methods of estimation of carbohydrates, especially in plant extracts. A new method for the estimation of maltose in presence of other sugars. Jour. Agr. Sci. **5**: 437–468. 1913.

6. EMERSON, R., and LEWIS, C. M. Carbon dioxide exchange and the measurement of the quantum yield of photosynthesis. Amer. Jour. Bot. **28**: 789–804. 1941.

7. HEINZE, P. H., and MURNEEK, A. E. Comparative accuracy and efficiency in determination of carbohydrates in plant material. Univ. Missouri Agr. Exp. Sta. Research Bull. 314. 1940.

8. KOSTYTSCHEW, A. Studien über Photosynthese. I. Das Verhältnis CO_2/O_2 bei der Kohlensäureassimilation. Ber. d. bot. Gesell. **39**: 319–328. 1921.

9. KRASCHENINNIKOV, TH. Accumulation of solar energy in plants. Moscow, p. 55, 1901.

10. MAQUENNE, L., and DEMOUSSY, E. Échanges gaseux des plantes vertes. Gauthier-Villars. Paris, p. 128. 1913.

11. RUBEN, S., HASSID, W. Z., and KAMEN, M. D. Radioactive carbon in the study of photosynthesis. Jour. Amer. Chem. Soc. **61**: 661–663. 1939.

12. SAPOSCHNIKOFF, W. Bildung und Wanderung der Kohlenhydrate in den Laubblättern. Ber. d. bot. Ges. **8**: 233–242. 1890.

13. SMITH, J. H. C. The absorption of carbon dioxide by unilluminated leaves. Plant Physiol. **15**: 183–284. 1940.

14. SPOEHR, H. A. Photosynthesis. The Chemical Catalogue Co., New York, 1926. a, pp. 87–94; b, p. 221; c, p. 250.

15. —————. The culture of albino maize. Plant Physiol. **17**: 397–410. 1942.

16. —————, and MILNER, H. W. Starch dissolution and amylolytic activity in leaves. Proc. Amer. Philos. Soc. **81**: 37–78. 1939.

17. STILES, W. Photosynthesis. Longmans, Green and Co., London, pp. 147–151. 1925.

18. VAN SLYKE, D. D., and FOLCH, J. Manometric carbon determination. Jour. Biol. Chem. **136**: 509–541. 1940.

RÔLE OF ETHER SOLUBLE ORGANIC ACIDS IN THE CATION-ANION BALANCE IN PLANTS[1]

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(WITH SIX FIGURES)

Introduction

Factors that influence salt absorption by plants have long been a subject of study. Only recently, however, (**3, 4, 7, 8, 11, 16, 20, 23**), have the effects of certain metabolic processes in the plant been recognized as intimately connected with salt absorption and accumulation.

The present investigation has been concerned with the cation-anion balance in several species of crop plants with special reference to the rôle of ether soluble organic acids. Prior to the work of PUCHER, VICKERY, and WAKEMAN (**13, 14, 15**), on the quantitative determination of organic acids in plant material, lack of accurate methods had seriously curtailed investigation along these lines. A study has been made of the selective capacity of the various species with respect to inorganic ions; of the quantity and kinds of organic acids in the different species grown in similar culture medium; and of the correlation between inorganic cations and ether soluble organic acids in the different species as a group.

Materials and methods

CULTURE METHODS

Twelve species of plants were chosen for this investigation: spinach, variety Summer Savoy (*Spinacea oleracea* L.); beets, variety Early Superb (*Beta vulgaris* L.); wheat, variety Leapland (*Triticum aestivum* L.); Kentucky blue grass (*Poa pratensis* L.); alfalfa (*Medicago sativa* L.); lima bean (*Phaseolus limensis* Macf.); peas, variety Alaska (*Pisum sativum* L.); soybean, variety Biloxi (*Glycine soja* Sieb. and Zucc.); buckwheat (*Fagopyrum esculentum* Gaertn.); lettuce, variety Grand Rapids (*Lactuca sativa* L.); cantaloupe, variety White Seeded Pink Meat (*Cumcumis melo* L.); and tomato, variety Break of Day (*Lycopersicon esculentum* Mill.). The seeds of the various species were planted in sand and when the plants had attained sufficient height they were thinned to a few uniform plants per pot. Six pots were allotted to each species of plant.

The nutrient solution used had the following composition: 0.005 M Ca $(NO_3)_2$, 0.0025 M KH_2PO_4, and 0.0025 M $MgSO_4$. The elements Cu, Mn, Zn, B, and Fe were used in the usual small quantities to supplement the three main salts in the nutrient solution. This solution was applied to all plants at the rate of approximately 1 liter per crock, per 24 hours.

A modification of the SHIVE and STAHL capillary drip method (**19**) was

used to apply the nutrient solution.   The modification consisted of a series of enamel basins, usually 3, connected by siphons and constantly supplied by solution from an inverted 18-liter bottle.   The basins were placed on a shelf between twin rows of coffee urn liners filled with sand; these crocks permit good drainage.   The shelf was 2 to 3 inches higher than the tops of the crocks.   The basins were covered with round lids cut from ·Celotex and painted with asphalt paint to prevent water absorption.   Four crocks were supplied with nutrient from each basin by inserting capillary tubes into notches cut on the under side of the lids.   Adjustment of the capillary tubes was facilitated by adjustable metal supports fastened to the shelf near the basins.   The inverted 18-liter bottle maintained a constant head of pressure and insured uniform delivery of nutrient solution at all times.   After the initial adjustment of the capillaries, which was done with the aid of a stop-watch, the only thing required was to keep the 18-liter reservoir filled.

The 1940 crop was planted the latter part of September and all plants had been harvested by the end of November.   In 1941 the experiment was repeated.   This crop was planted the latter part of January and all plants had been harvested by the middle of April, with the exception of cantaloupe. The cantaloupe was not planted until April.   The night temperature of the greenhouse was between 65° and 70° F.

These experiments were concerned only with the vegetative phase of growth.   All plants were harvested before they blossomed or, in cases where blossoms appeared before sufficient vegetative growth had been attained, the blossoms were pinched off.

TISSUE PREPARATION AND ANALYTICAL METHODS

All samples were taken between 9 : 00 and 11 : 00 A.M.   Each set of plants was divided into two equal lots and the fresh weight obtained immediately. Where possible both lots were divided into stems and leaves, petioles being included with the stems.   The leaves and stems of one lot were placed in separate quart fruit jars, the lid put on tightly, and jars placed in a refrigerator at $-15°$ C.   The leaves and stems of the other lot were placed in separate wire baskets, dried in a hot air oven at 70° C., ground to pass a 60-mesh sieve, then stored in aluminum boxes until ready for analysis.   The samples put up for juice analysis were frozen at $-15°$ C. for at least 12 hours.   After this time they were allowed to stand at room temperature until completely thawed.   This usually required 3 to 4 hours.   The material was then placed in a stout muslin cloth, subjected to 10,000 pounds pressure per square inch in a Carver hydraulic press, and the juice collected.   The methods of the Association of Official Agricultural Chemists (1) were used for the final determination of calcium, magnesium, potassium, sulphur, and nitric acid.

MOISTURE.—Immediately after the plants were cut, duplicate 15–20 gram samples of leaves and stems were snipped into tared aluminum boxes.   The samples were dried to constant weight in a vacuum oven at 65° C. and 2–3 cm. pressure.   All results are based on dry tissue.

ASHING.—Duplicate ¾-gram samples of the dried, finely ground material were weighed into tared platinum crucibles. The material was moistened with 25 drops of sulphuric acid (1–10), dried in an oven at 95° C., then placed in a muffle furnace and ashed 4 hours at 600° C. The sulphuric acid was necessary to avoid explosion of the material while ashing.

CALCIUM.—The residue from the ash determination was used for the determination of calcium. The method used was identical with that employed for the analysis of calcium in the juice, except that the volume of the ash solution was 50 ml.

MAGNESIUM.—The filtrate and washings from the calcium determination did not contain sufficient magnesium to permit an accurate determination of this constituent. A larger aliquot, 20 ml., was used. This was treated in the same way as the calcium determination and magnesium determined on the filtrate and washings.

POTASSIUM.—Duplicate 10-ml. aliquots of the ash solution were used for the determination of potassium. The rapid official method (1) was used.

PHOSPHORUS AND SULPHUR.—A separate ashing was necessary for the determination of phosphorus and sulphur. Duplicate 1.0-gram samples were weighed into large porcelain crucibles and ashed by the magesium nitrate method (1). Phosphorus in the ash was determined by the method of FISKE and SUBBAROW (6). An Aminco photometer was used for the comparisons. A standardization curve was constructed, using varying concentrations of pure KH_2PO_4. Sulphur in the ash solution was determined by the official method (1).

TOTAL NON-VOLATILE ORGANIC ACIDS, OXALIC AND CITRIC ACIDS.—The methods used were those of PUCHER, VICKERY, and WAKEMAN (13, 14).

MALIC ACID.—The uranium acetate method of DUNBAR and BACON (5), modified by VICKERY and PUCHER (21) was used. Admittedly this method is not as good as the one developed later by PUCHER, VICKERY, and WAKEMAN (15) but the time saved justified its use.

NITRIC ACID.—The method used for the extraction of the organic acids also extracted the nitric acid quantitatively. A suitable aliquot was reduced, distilled, and Nesslerized (1). Readings were taken in an Aminco photometer and the quantity of nitrogen read from a standardization curve.

SAP ANALYSIS

The determination of sap soluble constituents in the extracted juice is based on the work of SAYRE and MORRIS (17, 18).

TOTAL SOLIDS.—Duplicate 2-ml. samples of the juice were dried in a hot air oven at 70° C., then placed in a vacuum oven at 65° C. and 2 to 3 cm. pressure until constant weights were obtained.

OXALIC ACID.—Aliquots of 5 to 10 ml. of juice, depending upon the oxalic acid content, were pipetted into 25-ml. volumetric flasks and made to volume. Duplicate 10-ml. aliquots of the diluted juice were measured into 100-ml. beakers and 0.5 normal HCl added until slightly acid to Congo red. This

was allowed to stand overnight and any material that flocculated was filtered off on an asbestos mat. The coagulum was washed 2 or 3 times with small amounts of water slightly acidified with HCl. From this point the method was identical with that used by PUCHER, VICKERY, and WAKEMAN (**13**) to determine oxalic acid in the ether extract of dried tissue.

CALCIUM.—Duplicate 10-ml. samples of the juice were measured into small porcelain crucibles and dried in a hot air oven at 70° C. The material

TABLE I

INORGANIC CATIONS, 1940 CROP

PLANT		MILLIEQUIVALENTS PER 100 GRAMS OF DRY TISSUE				
		TOTAL MAGNESIUM	TOTAL POTASSIUM	TOTAL CALCIUM	SOLUBLE CALCIUM	SOLUBLE CALCIUM, PERCENTAGE OF TOTAL
		m. eq.	*m. eq.*	*m. eq.*	*m. eq.*	%
Lima bean	Leaves	74.0	111.2	117.2	80.5	68.7
	Stems	64.6	167.3	81.7	57.5	70.5
Peas	Leaves	52.6	78.9	128.0		
	Stems	34.5	115.1	59.0		
Alfalfa	Leaves	56.7	80.6	132.6	69.6	52.5
	Stems	42.8	92.7	45.6	16.3	35.8
Soybean	Leaves	93.5	88.5	112.3	55.8	49.7
	Stems	85.5	146.1	80.3	38.8	48.3
Beets	Leaves	240.6	176.6	99.0	2.3	2.4
	Petioles	92.9	348.5	27.8	4.7	17.2
Spinach	Leaves	165.3	186.5	127.6	1.3	1.0
	Petioles	103.0	333.7	74.3	2.0	2.8
Buckwheat	Leaves	188.5	61.9	146.6	4.8	3.3
	Stems	110.8	243.0	105.6	13.6	12.9
Bluegrass	Leaves	52.6	155.1	24.6	20.3	82.6
Wheat	Leaves	52.1	191.5	22.6	17.0	75.3
Tomato	Leaves	114.3	102.0	183.0	91.0	49.8
	Stems	129.4	228.0	104.3	33.8	32.4
Lettuce	Leaves	59.2	216.9	81.6	48.7	59.6
Cantaloupe	Leaves	125.0	96.3	291.3	45.7	15.7
	Stems	93.2	216.9	79.6	46.0	57.8

was moistened with a few drops of sulphuric acid (1 : 10) and heated gently over a low flame until thoroughly charred. The crucibles were then placed in a muffle furnace and ashed for 4 hours at 600° C. The addition of sulphuric acid was necessary to eliminate explosion of the material during ashing.

The ash was dissolved in dilute HCl, taken to dryness to dehydrate any silica, redissolved in dilute HCl and made to a volume of 25 ml. Calcium was determined on 10-ml. aliquots of this solution by the official micro method (**1**).

Results

The plants from the 1940 and 1941 crops were quite comparable with respect to age, height, and fresh weight, with the exception of cantaloupe.

The 1940 crop of cantaloupe was grown in the late fall when light intensity was low and consequently there was less growth than in the 1941 crop which was grown in April and May when light intensity was increasing. Regardless of the difference in the amount of growth of the two crops, analyses based on dry weight showed them to be quite comparable. The absolute values for age, height, and fresh weight are not recorded as they were of importance only in comparing the growth of the two crops.
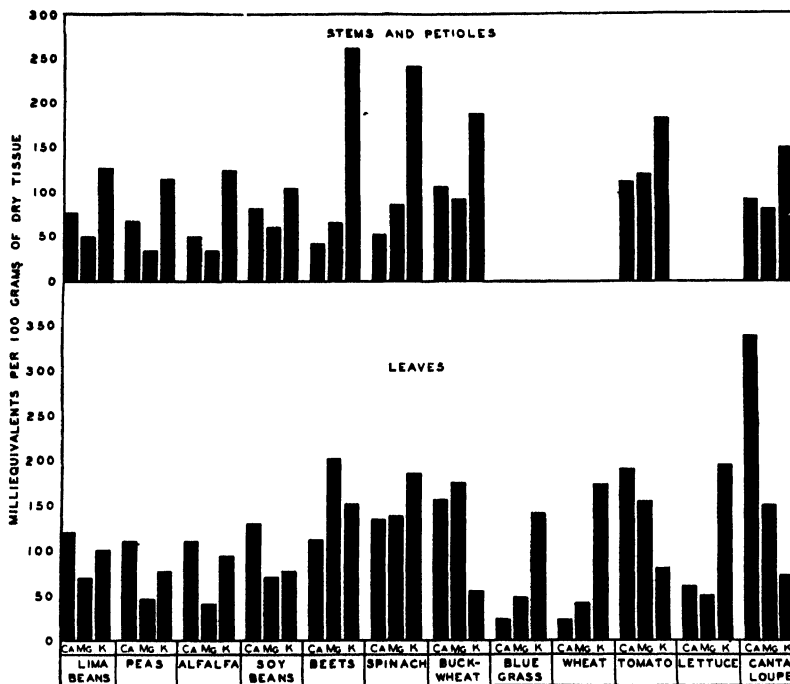
FIG. 1. Distribution of Ca, Mg, and K in leaves and stems of the species tested (1941 crop).

INORGANIC CATIONS AND ANIONS

Table I gives the inorganic cations in milliequivalents per 100 grams of dry tissue in the different plants for the 1940 crop and table II the inorganic cations and anions for the 1941 crop. The inorganic anions were not determined in the 1940 crop. It can be seen that the inorganic ion content is quite comparable in the two crops. The data on the cations of the 1941 crop are shown more clearly in fiigure 1. Figure 2 shows graphically the distribution of calcium. Since the 1940 crop was similar it is not included.

It is clearly shown that when different species of plants are all grown under similar conditions of nutrient supply they take up inorganic ions in varying proportions according to inherent characteristics of the plant. It should be noted that even plants in the same families all tend to accumulate cations in the same relative proportions (legumes: soy beans, lima beans, alfalfa, and peas; the Chenopodiaceae: beets and spinach; the grasses: bluegrass and wheat. These results corroborate those of NEWTON (9).
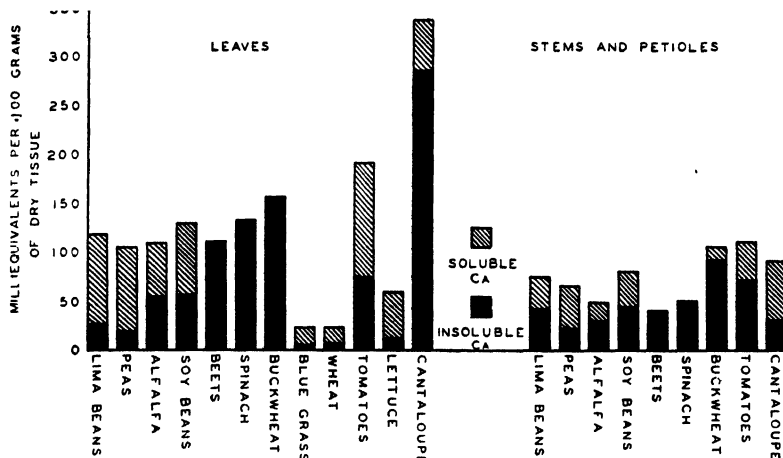
FIG. 2.   Relative amounts of soluble and insoluble calcium in leaves and stems of the species tested (1941 crop).

Calcium and magnesium tend to accumulate in greater quantities in the leaf blade tissue than in the stems and petioles.   The reverse is true of potas-

TABLE II

INORGANIC ANIONS AND CATIONS, 1941 CROP

PLANT		MILLIEQUIVALENTS PER 100 GRAMS OF DRY TISSUE							SOLUBLE CALCIUM, PERCENTAGE OF TOTAL
		TOTAL NO$_3$	TOTAL SO$^=_4$	TOTAL H$_2$PO$_4$	TOTAL MAG-NESIUM	TOTAL POTAS-SIUM	TOTAL CAL-CIUM	SOLU-BLE CAL-CIUM	
		m. eq.	m. eq.	m. eq.	m. eq.	m. eq.	m. eq.	m. eq.	%
Lima bean	Leaves	28.4	18.5	13.1	69.6	98.9	118.3	90.8	76.8
	Stems	32.3	9.5	15.2	48.5	125.7	75.0	31.5	42.1
Peas	Leaves	19.5	25.9	23.9	47.8	77.8	105.5	86.5	82.1
	Stems	48.7	12.8	15.3	33.7	114.4	66.0	43.1	65.3
Alfalfa	Leaves	24.6	34.4	19.4	41.4	93.4	109.0	53.9	49.4
	Stems	29.6	15.1	21.8	33.4	123.6	49.7	18.4	37.3
Soybean	Leaves	15.0	23.0	24.9	71.0	76.6	128.7	71.6	55.7
	Stems	32.1	23.3	23.6	58.1	104.2	81.0	36.0	44.4
Beets	Leaves	22.3	30.0	24.9	201.8	151.7	112.0	1.3	1.2
	Petioles	157.5	12.4	15.2	65.3	261.0	42.3	2.7	8.9
Spinach	Leaves	40.4	30.5	39.7	137.3	186.7	.133.7	1.1	0.9
	Petioles	100.3	22.2	37.9	85.2	242.1	51.3	2.6	5.2
Buckwheat	Leaves	17.7	21.0	27.1	174.9	57.8	157.0	2.8	1.8
	Stems	162.5	12.2	22.1	92.1	186.7	106.0	13.5	12.7
Bluegrass	Leaves	74.3	26.7	23.4	47.7	142.2	23.0	17.0	75.7
Wheat	Leaves	76.9	30.0	35.7	41.7	173.1	22.7	15.0	66.7
Tomato	Leaves	20.4	91.8	30.0	105.3	82.4	191.7	116.8	60.9
	Stems	106.4	15.2	29.7	119.3	181.4	112.0	39.7	35.3
Lettuce	Leaves	54.3	24.8	26.3	50.5	195.2	60.7	47.5	78.5
Cantaloupe ·	Leaves	44.3	72.4	26.9	148.6	72.6	336.7	51.0	15.2
	Stems	120.7	27.4	26.1	81.1	148.0	91.7	59.8	65.5

sium.   Much larger quantities of this element accumulate in the stems and petioles than in the leaf blades.   The exceedingly high amount of calcium present in cantaloupe leaves is of special interest and will be considered later.   The soluble calcium as percentage of the total ranges from 70 to 80 per cent. in some plants and down to 1 per cent. in others.

ORGANIC ACIDS

Tables III and IV give the values of the various organic acids in milliequivalents per 100 grams of dry tissue in the two crops, 1940 and 1941.

TABLE III

ORGANIC ACIDS, 1940 CROP

PLANT		MILLIEQUIVALENTS PER 100 GRAMS OF DRY TISSUE					UNKNOWN AS PERCENTAGE OF TOTAL
		TOTAL ACIDS	OXALIC	MALIC	CITRIC	UNKNOWN ACIDS	
		m. eq.	m. eq.	m. eq.	m. eq.	m. eq.	%
Lima bean	Leaves	237.2	30.2	24.9	38.5	143.6	60.5
	Stems	259.3	29.6	30.2	32.2	167.3	64.5
Peas	Leaves	255.7	17.0	56.1	86.0	96.6	37.8
	Stems	219.6	6.0	47.0	56.0	110.6	50.4
Alfalfa	Leaves	199.5	30.0	24.9	22.9	121.7	61.0
	Stems	166.8	9.8	16.8	10.9	129.3	77.5
Soybean	Leaves	246.1	17.4	1.3	58.0	169.4	68.8
	Stems	235.5	21.8	26.2	20.6	166.9	70.9
Beets	Leaves	405.9	298.0	21.2	20.6	66.1	16.3
	Petioles	201.8	86.6	40.0	22.4	52.8	26.2
Spinach	Leaves	362.1	280.4	6.5	3.7	71.5	19.7
	Petioles	238.5	150.9	16.8	4.9	65.9	27.6
Buckwheat	Leaves	353.6	253.8	15.8	26.4	57.6	16.3
	Stems	207.2	109.2	16.8	13.4	67.8	32.7
Bluegrass	Leaves	142.7	0.0	7.7	26.3	108.7	76.2
Wheat	Leaves	133.5	0.0	16.4	6.5	110.6	82.8
Tomato	Leaves	239.7	69.3	30.9	53.8	85.7	35.7
	Stems	220.5	82.1	52.7	10.7	75.0	34.0
Lettuce	Leaves	197.2	2.3	72.1	18.6	104.2	52.8
Cantaloupe	Leaves	85.6	0.0	22.2	34.0	29.4	34.3
	Stems	129.7	0.0	53.7	10.7	65.3	50.3

A word of warning is necessary when comparing the data in this experiment with data from plants grown under different conditions.   As CLARK (4) and WADLEIGH and SHIVE (23) have shown and as VICKERY and PUCHER (22) have stated, the kinds and amounts of organic acids occurring in plants are profoundly influenced by the form in which nitrogen is supplied to the plant.

It is evident that in many cases it is possible to account for only a small amount of the total organic acidity as oxalic, malic, and citric acids.   In wheat and blue grass the unknown organic acids make up 70 to 80 per cent. of the total; in beets, spinach, and buckwheat 70 to 85 per cent. of the total acids can be accounted for.   Data of this nature clearly show that there is

still much to be done in the way of developing suitable methods for the determination of the acids of the unknown group.

CATION-ANION BALANCE

PUCHER, VICKERY, and WAKEMAN (16) have shown in tobacco grown under controlled fertilizer conditions a large excess of positive ions over inorganic anions, and this excess is balanced by the ether soluble organic acids. An attempt has been made in the present investigation to determine if this is a common phenomenon among plants in general.

TABLE IV

ORGANIC ACIDS, 1941 CROP

PLANT		MILLIEQUIVALENTS PER 100 GRAMS OF DRY TISSUE					UNKNOWN AS PERCENTAGE OF TOTAL
		TOTAL ACIDS	OXALIC	MALIC	CITRIC	UNKNOWN ACIDS	
		m. eq.	m. eq.	m. eq.	m. eq.	m. eq.	%
Lima bean	Leaves	249.7	16.9	65.8	48.6	118.4	47.4
	Stems	171.1	31.1	24.5	33.1	82.4	48.2
Peas	Leaves	212.9	15.1	42.6	80.8	74.4	34.9
	Stems	181.0	7.3	46.5	34.5	92.7	51.2
Alfalfa	Leaves	185.7	14.1	21.8	14.5	135.3	72.9
	Stems	140.7	4.9	30.9	8.4	96.5	68.6
Soybean	Leaves	248.7	15.9	10.6	104.6	117.6	47.3
	Stems	177.0	18.9	10.9	26.8	120.4	68.0
Beets	Leaves	427.5	322.9	13.4	32.0	59.2	13.8
	Petioles	203.1	97.8	28.9	24.8	51.6	25.4
Spinach	Leaves	380.4	309.0	8.9	10.5	52.0	13.7
	Petioles	226.2	110.2	60.1	9.6	46.3	20.5
Buckwheat	Leaves	360.1	265.7	14.1	13.8	66.5	18.5
	Stems	205.6	110.4	34.1	6.6	54.5	26.5
Bluegrass	Leaves	108.5	0.0	9.1	27.8	71.6	66.0
Wheat	Leaves	122.1	0.0	18.3	9.6	94.2	77.2
Tomato	Leaves	269.8	45.2	61.1	74.4	89.1	33.0
	Stems	231.6	81.3	89.3	12.3	48.7	21.0
Lettuce	Leaves	220.8	1.4	109.0	32.2	78.2	35.4
Cantaloupe	Leaves	99.2	0.0	26.2	19.4	53.6	54.0
	Stems	119.4	0.0	54.4	5.9	59.1	49.5

In the analyses no attempt was made to fractionate organic and inorganic sulphur and phosphorus. In the calculations of milliequivalents of anions all sulphur was considered as sulphate and all phosphorus as diacid phosphate. This is not strictly true, as phosphorus and sulphur are known to enter into many organic compounds as proteins, phosphatides, hexose phosphates, etc. It seems safe to assume, however, that the major portions of these elements are in the inorganic form in the plant. All phosphorus was calculated as the monophosphate ion as this is practically the only form in which it can exist at the pH characteristic of the sap of the plants tested.

Table V presents data showing the relationship between the excess inorganic cations and ether soluble organic acids in the leaves and stems. Figure 3 presents the data of table V in graphic form.
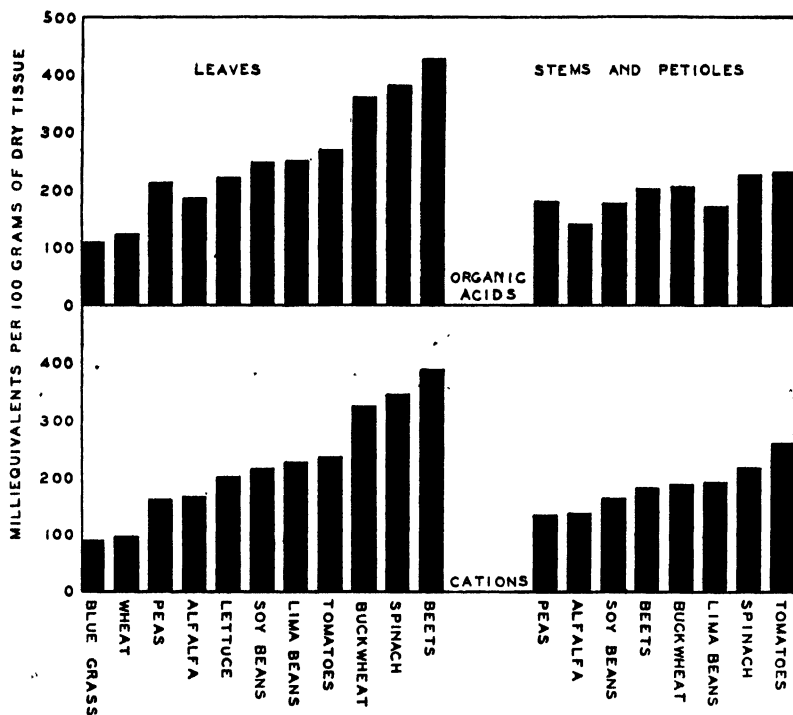
FIG. 3. Correlation between excess cations and total organic acids in leaves and stems of several species (1941 crop).

As far as the authors are aware these are the first data to show that inorganic cations and ether soluble organic acids are positively correlated in

TABLE V

CORRELATION BETWEEN EXCESS INORGANIC CATIONS AND TOTAL ORGANIC ACIDS, 1941 CROP

PLANT	MILLIEQUIVALENTS PER 100 GRAMS OF DRY TISSUE							
	LEAVES				STEMS AND PETIOLES			
	TOTAL Ca, Mg, K	TOTAL ANI-ONS*	EXCESS CATI-ONS†	TOTAL ORGANIC ACIDS	TOTAL Ca, Mg, K	TOTAL ANI-ONS*	EXCESS CATI-ONS†	TOTAL ORGANIC ACIDS
	m. eq.	m. eq.	m. eq.	m. eq.	m. eq.	m. eq.	m. eq.	m. eq.
Lima bean	286.8	59.9	226.9	249.7	249.2	57.0	192.2	171.1
Peas	231.2	69.2	162.0	212.9	214.1	76.9	137.2	181.0
Alfalfa	243.8	78.4	165.4	185.7	205.7	66.5	139.2	140.7
Soybean	276.2	62.9	213.3	248.7	243.3	79.0	164.3	177.0
Beets	465.5	77.2	388.3	427.5	368.5	185.0	183.5	203.1
Spinach	457.7	110.5	347.2	380.4	378.7	160.4	218.3	226.2
Buckwheat	389.7	65.8	323.9	360.1	384.8	196.8	188.0	205.6
Bluegrass	212.9	124.3	88.6	108.5	....	....	....	....
Wheat	237.0	142.6	94.7	122.1	....	....	....	....
Tomato	379.2	142.1	237.1	269.8	412.7	151.3	261.4	231.6
Lettuce	·306.3	105.4	200.9	220.8	....	....	....	....
Cantaloupe	557.9	143.6	414.3	99.2	320.8	174.2	146.6	119.4

* NO_3, $SO_4^=$, $H_2PO_4^-$.
† Total cations minus inorganic anions.

a series of different species of plants. The correlation in the leaf blade tissue is exceedingly high (+ 0.996), that in the stem and petiole tissue not as high (+ 0.798).

It will be noted in table V, that the cantaloupe plant has the highest cation content, but the lowest organic acid content, of any of the species tested. The exceedingly high amount of calcium present in the leaves of cantaloupe is rather unusual. Approximately 85 per cent. is insoluble but

FIG. 4. Comparison of the amounts of oxalic and citric acid with the amounts of different cations occurring in the plants tested.

it is not obvious in what combination it exists. There is no oxalic acid in the cantaloupe plant so the possibility of insoluble calcium oxalate is excluded. The pH 7.4 to 7.6 of the expressed sap is unusually high. This immediately suggests the possibility that part of the cations are tied up as carbonate and bicarbonate. Preliminary experiments, however, do not indicate a very large amount of CO_2 in the sap. Under more careful conditions this may not prove to be the case. Work is now in progress to deter-

mine what substances other than phosphate, sulphate, nitrate, and organic acids are binding the large quantities of cations in this plant. ILJIN (8) found, in general, that soluble calcium in plants was positively correlated with malic and citric acid content. PIATNITSKY (11) found that excised leaves of adult tobacco plants accumulated citric acid when exposed to a solution of $MgCl_2$. The increase in citric acid was nearly equivalent to the decrease in malic acid.
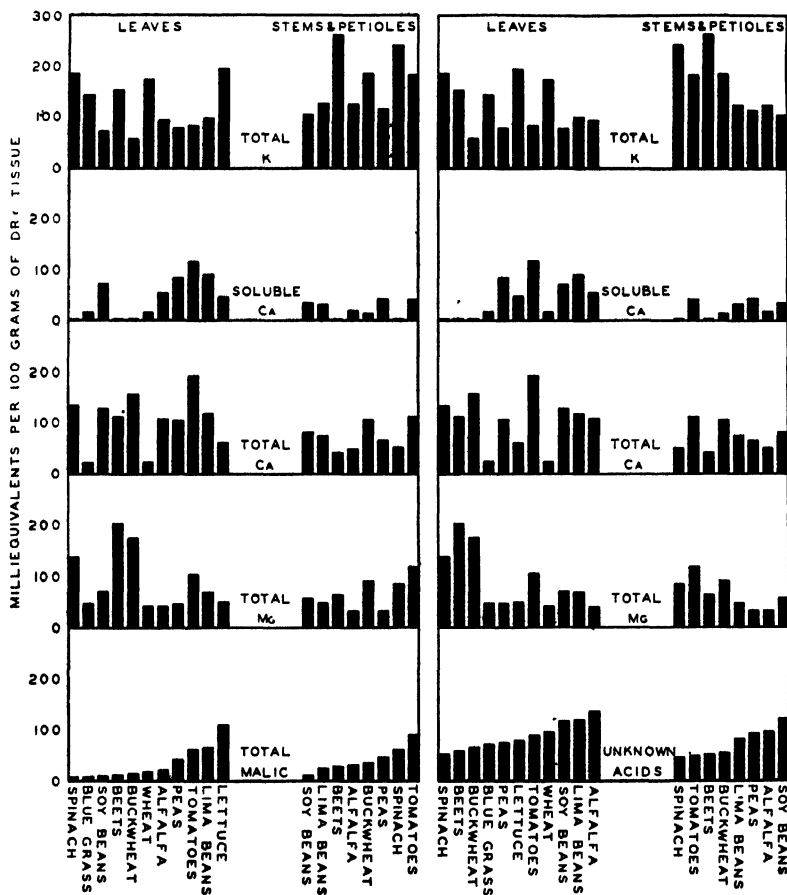
FIG. 5. Comparison of the amounts of malic acid and acids of the unknown group with the amounts of different cations occurring in the plants tested (1941 crop).

Figures 4 and 5 compare graphically the amounts of determined organic acids as well as the unknown group with the amounts of cations found in the various plants. No striking correlations are evident. Malic and citric acids are found to be somewhat correlated with soluble calcium, particularly in the leaf tissue. Citric acid and those acids of the unknown group show a small negative correlation with total magnesium content in the stems and petioles.

In spinach, beets, and buckwheat a relatively large proportion of the

organic acids could be accounted for principally as oxalic acid. Figure 4 shows that in the leaves of these plants total calcium decreases as total oxalic acid increases. Total magnesium content is considerably higher in these plants than in the others, indicating enhanced magnesium absorption.

Results of the present investigation indicate that those plants which produce no oxalic acid have very small quantities of insoluble calcium (figures 4 and 6). The major portion of the calcium is in a sap soluble state. There are exceptions to this, such as cantaloupe. In the plants that produce some oxalic acid, but no large quantity, all of the oxalic acid is precipitated, presumably as calcium oxalate. The major portion of the calcium, however, is
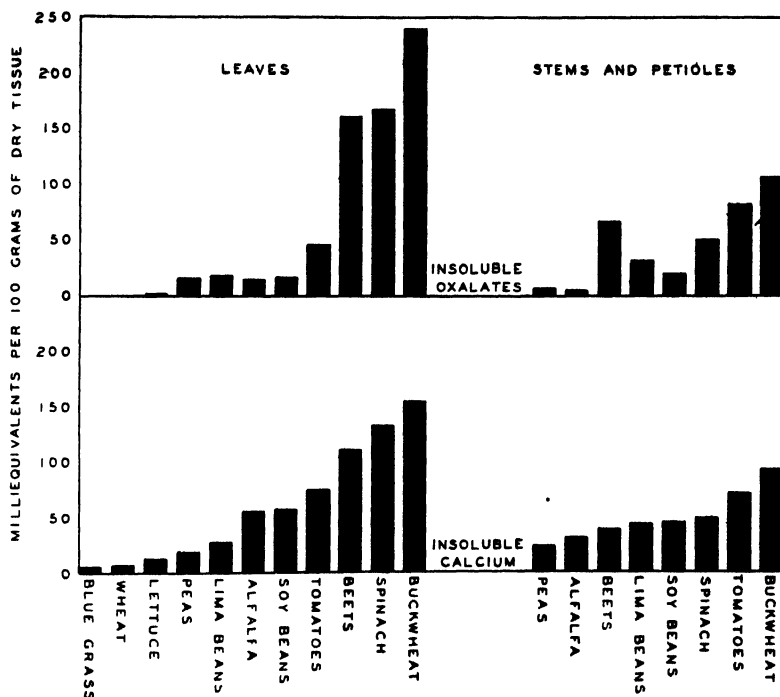
FIG. 6. Relationship between insoluble calcium and insoluble oxalates in leaves and stems of several species tested (1941 crop).

still in a sap soluble state. Those plants (beets, spinach, and buckwheat), which produce large quantities of oxalic acid have practically all of the calcium in an insoluble state, only traces of sap soluble calcium occurring. In addition these plants have considerable quantities of oxalates dissolved in the cell sap. According to this, the picture ranges from those plants with no oxalic acid and a relatively large proportion of sap soluble calcium to those plants with large quantities of oxalic acid and little or no soluble calcium.

Table VI shows the high positive correlation between insoluble calcium and insoluble oxalates. Figure 6 presents the same data in graphic form for the 1941 crop. It will be noted that in the three plants producing the larg-

TABLE VI

CORRELATION BETWEEN INSOLUBLE OXALIC ACID AND INSOLUBLE CALCIUM,
1940 CROP

PLANT	MILLIEQUIVALENTS PER 100 GRAMS OF DRY TISSUE			
	LEAVES		STEMS AND PETIOLES	
	INSOLUBLE OXALIC ACID	INSOLUBLE CALCIUM	INSOLUBLE OXALIC ACID	INSOLUBLE CALCIUM
	m. eq.	*m. eq.*	*m. eq.*	*m. eq.*
Lima bean	30.2	36.7	29.6	24.2
Peas				
Alfalfa	30.0	63.0	9.8	29.3
Soybean	17.4	56.5	21.8	41.5
Beets	209.8	96.7	37.9	23.1
Spinach	155.0	126.3	84.3	72.3
Buckwheat	242.9	141.8	109.2	92.0
Bluegrass	0.0	4.3		
Wheat	0.0	5.6		
Tomato	69.3	92.0	82.1	70.5
Lettuce	2.3	32.9		
	Correlation coefficient = + .886		Correlation coefficient = + .929	

1941 CROP

PLANT				
Lima bean	16.9	27.5	31.1	43.5
Peas	51.1	19.0	7.5	22.9
Alfalfa	14.1	55.1	4.9	31.3
Soybean	15.9	57.1	18.9	45.0
Beets	161.0	110.7	66.1	39.6
Spinach	167.4	132.6	49.4	48.7
Buckwheat	239.8	154.2	100.6	92.5
Bluegrass	0.0	6.0		
Wheat	0.0	7.7		
Tomato	45.2	74.9	81.3	72.3
Lettuce	1.4	13.2		
	Correlation coefficient = + .946		Correlation coefficient = + .884	

est quantities of oxalic acid, insoluble oxalates exceed the amount of insoluble calcium. Obviously some other cation besides calcium must exist in the form of an insoluble oxalate in the plant. Whenever juice of beets, spinach, or buckwheat is allowed to stand for a few hours a white crystalline precipitate appears. This has been identified as pure magnesium oxalate. Previous evidence has been obtained by the senior author (12) that magnesium is precipitated by oxalic acid. Magnesium oxalate is known to form a supersaturated solution rather easily and it appears that this is what happens in the plant. With an increased uptake of magnesium, however, the limits of supersaturation are bound to be exceeded and some of the magnesium oxalate will be precipitated.

Summary and conclusions

Twelve different species of plants were grown in the greenhouse under controlled solution culture and all plants received the same nutrient supply.

Chemical analyses were made with the idea of studying the cation-anion balance in the different species of plants.

Inorganic ions were found to be taken up in varying proportions according to inherent characteristics of the plant. Plants in the same family tended to accumulate ions in relatively the same proportions.

Data were obtained on the kinds and amounts of organic acids occurring in a variety of plants. In some plants the unknown fraction of organic acids made up 70 to 80 per cent. of the total, while in others the unknown fraction amounted to only 15 to 25 per cent.

In all of the plants studied, except cantaloupe, a large excess of inorganic cations over inorganic anions was found and when all plants were considered together this excess was found to be highly correlated with total ether soluble organic acids. Cantaloupe was the outstanding exception. It contained the largest amounts of cations but the smallest amounts of organic acids of any of the species tested.

In the leaves malic and citric acids showed a rather low positive correlation with soluble calcium. Citric acid and those acids of the unknown group showed a rather small negative correlation with total magnesium content in the stems and petioles.

Insoluble oxalates and insoluble calcium were found to be highly correlated when the plants were considered as a group. In three cases insoluble oxalic acid exceeded the amount of insoluble calcium. Evidence was cited that the additional insoluble oxalic acid was present as magnesium oxalate. Magnesium content increased with increased oxalic acid content.

Those plants with little or no oxalic acid had a large proportion of the calcium in a sap soluble state, while those plants high in oxalic acid had but traces of sap soluble calcium.

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LITERATURE CITED

1. ASSOCIATION OF OFFICIAL AGRICULTURE CHEMISTS. Methods of Analysis, Fourth Ed. 1935.
2. BENNET-CLARK, T. A. The role of organic acids in plant metabolism. Part I. New Phyt. **32**: 37–71. 1935.
3. CHANDLER, R. F. Certain relations between calcium and oxalate content of foliage of certain forest trees. Jour. Agr. Res. **55**: 393–398. 1937.
4. CLARK, HAROLD E. Effect of ammonium and of nitrate nitrogen on the composition of the tomato plant. Plant Physiol. **11**: 5–24. 1936.
5. DUNBAR, P. B., and BACON, R. E. Determination of malic acid. Ind. & Eng. Chem. **3**: 826–831. 1911.
6. FISKE, C. H., and SUBBAROW, Y. The colorimeter determination of phosphorus. Jour. Biol. Chem. **66**: 375–400. 1925.

7. HOAGLAND, D. R., and BROYER, T. C. Hydrogen-ion effects and the accumulation of salt by barley roots as influenced by metabolism. Amer. Jour. Bot. **27**: 173–185. 1940.

8. ILJIN, W. S. Calcium content in different plants and its influence on production of organic acids. Bull. Assoc. Russe Res. Sci. a Progue. Sect. Sci. Nat. et Math. **41**: 43–76. 1938.

9. NEWTON, J. D. The selective absorption of inorganic elements by various crop plants. Soil Sci. **26**: 85–91. 1928.

10. PARKER, F. W., and TRUOG, E. The relation between the calcium and nitrogen content of plants and the function of calcium. Soil Sci. **10**: 49–56. 1920.

11. PIATNITSKY, M. P. Effect of mineral salts on storage of citric acid by leaves of *Nicotiana rustica* and *Nicotiana glauca*. Compt. Rend. (Doklady) Acad. Sci. U. R. S. S. **29**: 59–61. 1940.

12. PIERCE, E. C. Master's thesis, University of New Hampshire.

13. PUCHER, G. W., VICKERY, H. B., and WAKEMAN, A. J. Determination of the acids of plant tissue. II. Total organic acids of tobacco leaf. Ind. Eng. Chem. Anal. Ed. **6**: 140–143. 1934.

14. ————————, ————————, and LEAVENWORTH, C. S. Determination of acids of plant tissue. III. Determination of citric acid. Ind. & Eng. Chem. Anal. Ed. **6**: 190–192. 1934.

15. ————————, ————————, and WAKEMAN, A. J. Determination of malic acid in plant tissue. Simultaneous determination of citric and malic acid. Ind. & Eng. Chem. Anal. Ed. **6**: 288–291. 1934.

16. ————————, ————————, and ————————. Relationship of the organic acids of tobacco to the inorganic basic constituents. Plant Physiol. **13**: 621–630. 1938.

17. SAYRE, J. D., and MORRIS, V. H. Use of expressed sap in physiologic studies of corn. Plant Physiol. **6**: 139–148. 1931.

18. ————————, and ————————. Use of expressed sap in determining the composition of corn tissue. Plant Physiol. **7**: 261–272. 1932.

19. SHIVE, J. W., and STAHL, A. L. Constant rates of continuous solution renewal for plants in water cultures. Bot. Gaz. **84**: 317–323. 1927.

20. ULRICH, A. Metabolism of non-volatile organic acids in excised barley roots as related to cation-anion balance during salt accumulation. Amer. Jour. Bot. **28**: 526–537. 1941.

21. VICKERY, H. B., and PUCHER, G. W. Chemical investigation of the tobacco plant. IV. The effect of the curing process on the organic acids of tobacco leaves. Connecticut Agr. Exp. Sta. Bull. 352. 1933.

22. ————————, and ————————. Organic acids of plants. Ann. Rev. Biochem. **9**: 529–544. 1940.

23. WADLEIGH, C. H., and SHIVE, J. W. Organic acid content of corn plants as influenced by pH of substrate and form of nitrogen supplied. Amer. Jour. Bot. **26**: 244–248. 1939.

AMOUNT AND DURATION OF GROWTH OF VARIOUS SPECIES OF TREE SEEDLINGS

PAUL J. KRAMER

(WITH FOUR FIGURES)

Introduction

This paper deals with the results of measurements of the length of growing season and amount of growth made by tree seedlings of eleven different species, including some species with northern and some with southern ranges. *The seedlings were grown together for three years under the same environmental conditions. The species used were balsam fir (*Abies balsamea* (L.) Mill.), loblolly pine (*Pinus taeda* L.), shortleaf pine (*P. echinata* Mill.), slash pine (*P. caribaea* Morelet), red pine (*P. resinosa* Ait.), white pine (*P. strobus* L.), white ash (*Fraxinus americana* L.), eastern red oak (*Quercus borealis maxima* (Marsh) Ashe), white oak (*Q. alba* L.), yellow poplar (*Liriodendron tulipifera* L.) and black walnut (*Juglans nigra* L.). The balsam fir, red, and white pine were obtained from the New York State College of Forestry at Syracuse and were three years old. The red and white oak were grown from seed in our greenhouses and were one year old; all of the other species were one-year-old stock from the state nursery at Clayton, North Carolina.

The red pine and balsam fir do not naturally extend as far south as North Carolina; and white pine, with a few exceptions, is restricted to the mountains in this region. The normal range of slash pine does not extend as far north as North Carolina, while loblolly and shortleaf pine occur both north and south of North Carolina. This combination of species gave an excellent opportunity to compare the behavior of conifers planted north and south of their natural ranges with that of conifers growing well inside their natural ranges. Durham is within the southeastern limit of distribution for black walnut and red oak, and the other three deciduous species extend from the Gulf to Canada; thus all of the deciduous species used in this experiment were within their natural range.

A number of studies of tree growth have been made in America but none of them include comparisons of most of the species dealt with in this study, and none of them include data on the growth of northern species when transplanted outside their normal range. No formal review of the literature will be made as the pertinent papers are cited in connection with various phases of the work. BALDWIN (1) has discussed most of the important papers.

Methods

Thirty trees of each species were planted in a rectangular plot of ground in rows two feet apart, the individual trees being 18 inches apart in the rows. The individuals of each species except black walnut were located at random

in the plot so that all species were exposed to any variations in soil moisture and fertility in different parts of the plot. The black walnut was obtained too late to be included in the randomized planting so it was planted in a row along one side of the plot and only 25 trees were grown. Stakes were driven into the ground beside each tree the first year and reference marks made by driving nails through the stakes at the ground level. The second and third years' measurements were made from small nails driven into the trees. Measurements were made to 0.5 cm. with a meter stick. At the end of the second growing season the white ash and yellow poplar were so large that they were beginning to shade the other species so they were removed.

Results

The results of this experiment are discussed under three headings: first, the relative amounts of shoot growth made by the various species in a growing season; second, the relative lengths of growing seasons of the various species; and third, a comparison of the length of growing season of certain species at Durham, North Carolina, and in New England.

RELATIVE GROWTH

Relative amounts of growth made by the various species in a season are shown graphically in figures 1 and 2. As would be expected there are very considerable species differences. Yellow poplar and white ash grew much more rapidly than red and white oak and black walnut. The third season black walnut grew somewhat more rapidly than the oaks. The white ash and yellow poplar had become so large by the end of the second season that they seriously shaded the smaller conifers so they were cut. Differences in growth rate between the coniferous species were also consistently large. Loblolly pine made the most growth every year, slash and shortleaf pine made about two-thirds as much growth as loblolly; red and white pine made only about one-sixth as much growth as loblolly pine. The behavior of the pines in this experiment is in accord with their behavior in experimental plantations on the Duke Forest (14). Eight years after planting, the average heights of trees in adjacent plantations set out the same winter were: loblolly pine, 18.1; slash pine, 12.6; red pine, 2.3; and white pine, 3.8 feet. These trees were in adjacent plantations and their rate of growth might have been somewhat affected by variations in soil conditions; the trees in our experiment, however, were randomized among each other so that all species were exposed to similar soil conditions.

There were considerable differences in mortality among the seedlings of the various species, especially among the conifers (table I). The only deciduous species with high mortality was black walnut, 64 per cent. of the seedlings of this species having died by the end of the third year. Only three to ten per cent. of the seedlings of the other deciduous species died. Black walnut is rather difficult to transplant because of its large root system and furthermore it was not randomized with the other species, so one end

of the row may have been in relatively less favorable soil, thus increasing its mortality. The mortality among loblolly, shortleaf, and slash pine was only three to ten per cent., but 26 per cent. of the red pine and 46 per cent. of the white pine died during the three-year period. The highest mortality was in balsam fir of which 80 per cent. died the first season and .93 per cent. were dead by the end of the third season. In another nearby plot planted with seedlings from the same sources, and in most respects a replication of the one described in this paper, the mortality of red and white pine and balsam fir was comparable.
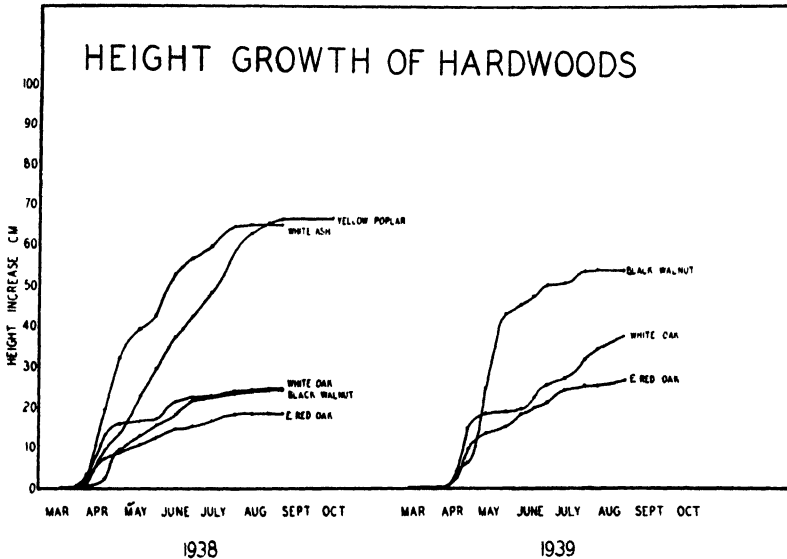
FIG. 1. Height growth of hardwoods during second and third seasons after planting. White ash and yellow poplar were removed at end of second season because they were overtopping other species.

LENGTH OF GROWING SEASON

Large differences existed between species with respect to length of growing season and course of growth during a season as well as with respect to the amount of growth made in a season. These data are summarized in table I. Figure 1 shows graphically the behavior of the hardwood species. All species resumed growth somewhat later the third season than the second season. Inspection of climatological data for Durham indicates that March, 1938, was about 6° F. warmer than average and March, 1939, about 3° F. warmer than average; it is doubtful, however, if this small difference in temperature can explain the difference in time of resumption of growth. Rainfall was adequate both springs. While white ash and yellow poplar behaved similarly in making more growth than any of the other hardwoods the manner in which this growth was distributed over the growing season was quite different in the two species (see figures 1 and 3). Both species resumed growth about April 1, but white ash made 50 per cent. of its growth

TABLE I

GROWTH MADE EACH MONTH EXPRESSED AS PERCENTAGE OF TOTAL SEASONS GROWTH. MORTALITY EXPRESSED AS PERCENTAGE OF ORIGINAL NUMBER OF SEEDLINGS DEAD AT END OF EACH SEASON

MONTH	WHITE ASH	YELLOW POPLAR	E. RED OAK		WHITE OAK		BLACK WALNUT		LOBLOLLY PINE		SHORTLEAF PINE		SLASH PINE		RED PINE		WHITE PINE		BALSAM FIR
	1938	1938	1938	1939	1938	1939	1938	1939	1938	1939	1938	1939	1938	1939	1938	1939	1938	1939	1938
	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%
March	0.0	0.0	4.0	0.0	6.0	0.0	2.0	0.0	5.0	2.0	2.0	3.0	6.0	4.0	16.0	8.0	13.0	0.0	0.0
April	49.0	20.0	45.0	45.0	57.0	45.0	38.0	18.0	21.0	14.0	24.0	16.0	22.0	19.0	66.0	55.0	67.0	65.0	37.0
May	20.0	25.0	21.0	17.0	6.0	5.0	26.0	65.0	15.0	27.0	13.0	22.0	14.0	25.0	8.0	32.0	10.0	32.0	47.0
June	20.0	21.0	15.0	21.0	21.0	20.0	24.0	11.0	21.0	23.0	17.0	19.0	17.0	17.0	5.0	2.0	4.0	3.0	3.0
July	11.0	23.0	15.0	12.0	7.0	20.0	7.0	6.0	15.0	14.0	16.0	14.0	17.0	14.0	3.0	0.0	6.0	0.0	12.0
August	1.0	10.0	0.0	5.0	3.0	10.0	3.0	0.0	14.0	13.0	20.0	16.0	16.0	12.0	2.0	3.0	0.0	0.0	0.0
September	0.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0	5.0	6.0	7.0	8.0	7.0	7.0	0.0	0.0	0.0	0.0	0.0
October	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.0	1.0	1.0	2.0	1.0	1.0	0.0	0.0	0.0	0.0	0.0
Growing season in days	130	160	125	135	140	135	150	115	210	210	200	200	200	200	140	150	127	115	120
Mortality	0.0	6.6	10.0	10.0	3.3	3.3	36.0	64.0	3.3	6.6	3.3	3.3	10.0	10.0	3.3	26.6	20.0	46.6	80.0

in April, slowed down in May and June, and ceased in late July or early August after a growing period of about 130 days. Yellow poplar had no peak month, but made about 20 per cent. of its growth each month from April to July inclusive, slowed down in August, and ceased in September after a growing period of 160 days. Red and white oak most nearly resembled white ash because they made about half the season's growth in April and had shorter growing seasons than yellow poplar. Even these species did not behave in exactly the same manner, however. Growth of eastern red oak gradually slowed down after the April peak and ceased in August while white oak had a peak in April, grew but little in May, but made rapid growth in June, then gradually slowed down and ceased growth in August. Compared to the oaks, black walnut started rather slowly in the spring, reached a peak in late April or May, and ceased growth late in July or in August
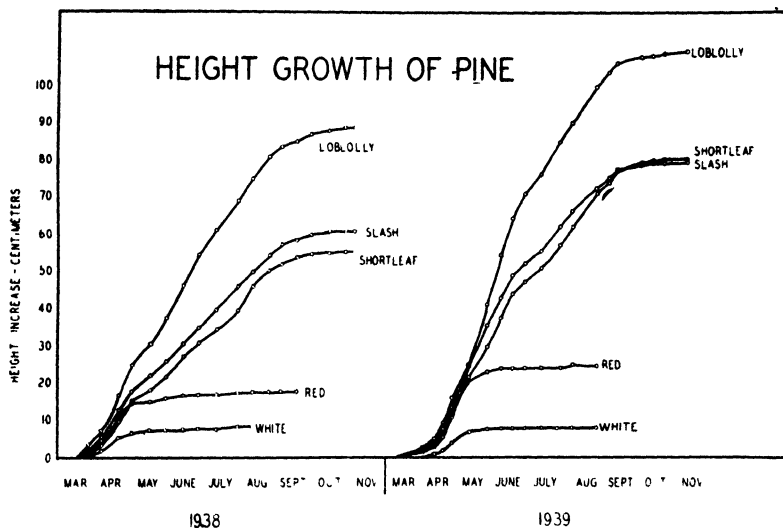
FIG. 2. Height growth of pines during two seasons.

after a growing season of 115 or more days. Individual trees ceased growth at intervals during the growing season, and even formed buds, then after a few weeks resumed growth again. This variation in the behavior of individual trees was most noticeable among the oaks, but occurred in all species, including the conifers.

The behavior of the pines is of particular interest because it provided a comparison of the length of growing season of the northern species, when grown in the south, with the southern species. The data for pine are shown graphically in figure 2. In general the northern species of pines made slightly more growth in March than the southern species though white pine did not resume growth until April 1 in 1939. The few surviving balsam fir seedlings resumed growth 7 to 10 days later than any other species studied. Both red and white pine made over 65 per cent. of their growth in April and over 90 per cent. of their growth by the end of May. White pine ceased

growth in late July while red pine made a small amount of growth in August. In contrast to the northern species, which made most of their growth in April and completely ceased to grow by July or August, loblolly, slash, and shortleaf pine had no well-defined peak in growth. All three species made measurable growth in March and made from 15 to 20 per cent. of the season's growth in every month from April to August, six to eight per cent. in September, and finally ceased growth early in October after growing seasons of 200 to 210 days. REED (17) in an earlier experiment at Durham, North Carolina, reported that loblolly pine resumed growth in late March and shortleaf pine in early April. Both species ceased growth about September 8, after a growing season of 170 days for loblolly, and 155 days for shortleaf pine. REED's trees were six years old and over six feet high and this difference in size may have some bearing on the difference in length of growing season.

<center>COMPARISON OF LENGTH OF GROWING SEASON IN NORTH
CAROLINA AND NEW ENGLAND</center>

Few data are available concerning the growth of hardwoods farther north except those of ILLICK (7) obtained at Mont Alto, Pennsylvania, and
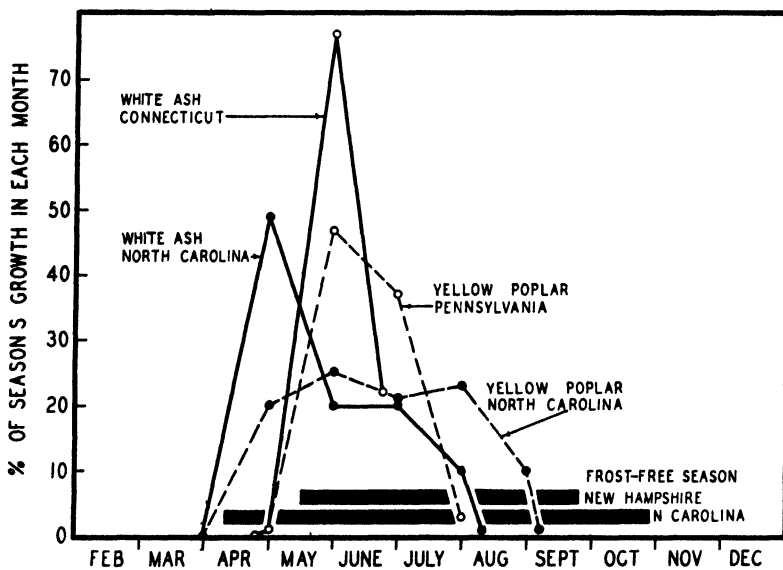
FIG. 3. Height growth of white ash and yellow poplar at Durham, North Carolina, and at northern stations. The data for white ash in Connecticut are from KIENHOLZ (11); the data for yellow poplar in Pennsylvania are from ILLICK (7).

those obtained by KIENHOLZ (11) in northwestern Connecticut. Their observations were made on trees which were older and larger than those measured in this study. Age and size of tree may have some effect on the length of the growing season but no definite information is available concerning the relation between length of growing season and age of tree. Figure 3 shows the length of growing season for white ash in North Carolina

and Connecticut and the length of growing season for yellow poplar in North Carolina and Pennsylvania as plotted from the data of KIENHOLZ and ILLICK. Study of figure 3 shows that, as would be expected, white ash and yellow poplar resumed growth nearly a month later at the northern stations and also ceased growth several weeks sooner. The time of maximum growth was also shifted about a month later at the more northern stations and the percentage of the season's growth made during the first month after the resumption of growth was much higher at the northern stations. The data for red oak give curves very similar to those for white ash at both stations. White ash and red oak grew twice as long in North Carolina as in Connecticut, and yellow poplar grew much longer in North Carolina than in Pennsylvania or Connecticut. It seems probable that the 95-day growing period
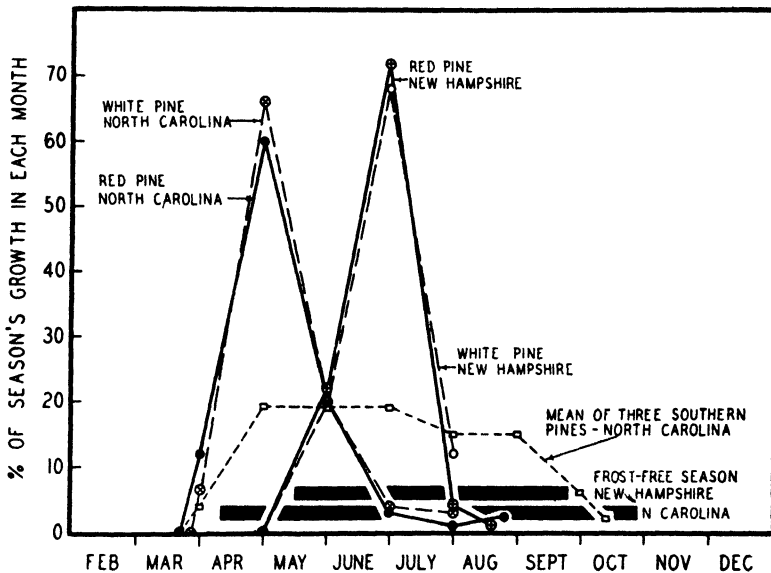
FIG. 4. Height growth of red and white pine at Durham, North Carolina, and Keene, New Hampshire. The New Hampshire data are from KIENHOLZ (10).

reported by ILLICK (7) in Pennsylvania does not include some late summer growth as KIENHOLZ (11) indicates that the growing season of yellow poplar in Connecticut is 105 to 110 days. JOHNSTON (9) reported that black, white, and post oak in the Missouri Ozarks had a growing season of only 19 days and made 90 per cent. of their growth in eleven days between April 27 and May 7. Those trees which averaged eight years in age and four feet in height behaved very differently from the oak in Connecticut and North Carolina. Our three- or four-year-old white oak seedlings grew from late March to late July and made about 50 per cent. of their total growth in April.

Several studies of the length of growing season of conifers have been made in various sections of the United States, but the most useful data for comparison seem to be those of KIENHOLZ (10), obtained in Southern New Hampshire. Figure 4 shows growth curves for red and white pine at Dur-

ham, North Carolina, and Keene, New Hampshire. Growth of both species started over a month later in New Hampshire and the peak came two months later than in North Carolina. The data of TRYON and FINN (**20**) from southern New York and FRIESNER (**6**) from southern Indiana indicate behavior at those places intermediate between that in North Carolina and in New Hampshire. Since the growing season naturally starts later and ends sooner at the northern stations than in North Carolina the growing season for red pine decreases from 145 days in North Carolina to 110 days in southern New Hampshire and the growing season of white pine decreases from 120 days in North Carolina to 100 days in New Hampshire. The 70-day growing season reported by ILLICK (**7**) for white pine in Pennsylvania seems abnormally short and probably does not include the small amount of growth made during the last month of the growing season. COOK (**4**) reported that red pine in Rensselaer County, N. Y., has an average growing season of only 53 days, but this also evidently does not include the small amount of late growth because TRYON and FINN (**20**) report a growing season of over 100 days only a short distance away. BALDWIN (**1**) found that white pine resumed growth about the same time at Ithaca, New York, Petersham, Massachusetts, and Berlin, New Hampshire, but was somewhat later at Cupsuptic Lake, Maine. The length of growing seasons seemed to be about the same, approximately 100 days, at all stations, including the one in Maine. FRIESNER (**6**) reported growth of red and white pine after the midsummer cessation of growth as late as October in Indiana. This possibly was related to an unusually warm and rainy autumn. The only data available on the other species of pine are those obtained by JOHNSTON (**9**) in the Missouri Ozarks where shortleaf pine grew from mid-April to early September, a total of 140 to 150 days compared to 200 days in North Carolina.

Discussion

Studies of tree growth under various environmental conditions should contribute to our knowledge of the factors determining the length of growing season and amount of growth made in a season. If we could obtain a more complete understanding of the factors determining the length of growing season and the time of maximum growth we might be able to control these factors advantageously in growing trees of better quality more rapidly. Two groups of factors interact in determining the amount of growth made by any tree. These are: (1), the hereditary potentialities as determined by its genetic composition; and (2), the complex of environmental factors to which the tree is exposed. The environment affects the amount and quality of growth by affecting the internal processes and conditions such as photosynthesis, respiration, accumulation of food, and water balance, within the limits of the hereditary potentialities of the individual tree or the species.

Inspection of figures 1 and 2 shows the very large genetic differences between species as expressed by differences in amount of growth and length of

growing season of seedlings grown in the same environment. Even so dras-
tic a change in environment as resulted from transferring red and white
pine from New England to North Carolina merely increased the absolute
lengths of their growing seasons, but did not change the relative lengths
nor the shapes of their growth curves (fig. 4). White ash and yellow poplar
have longer growing seasons in North Carolina than in Pennsylvania or
Connecticut but the essential differences between their growth curves exist
in both environments.

These results serve to emphasize that the length of growing season and
course of shoot growth of trees is relatively independent of normal fluctua-
tions in environmental factors during the growing season. Severe droughts
or excessively low temperatures may check growth, but the usual variations
in moisture and temperature have little effect. COOK (4) reported that only
unusual deficiency in rainfall retarded the growth of conifers and FRIESNER
(6) reported that heavy rainfall in June did not check the rapid decrease
in rate of growth of pines which occurred during that month. REED and
MacDOUGAL (18) state that periodicity in the growth of the orange tree can-
not be ascribed to environmental factors or depletion of reserve food. Ap-
parently the length of growing season and shape of the growth curve is
determined more by internal genetic factors than by the environment. It
is generally agreed that shoot growth is principally made at the expense of
stored food rather than from the products of current photosynthesis (3, 16).
BURGER (2) concluded that the amount of any one year's shoot growth is
largely dependent on the supply of reserve food accumulated the previous
season, and hence depends on the weather of the previous season more than
on the weather of the season in which growth is being measured. If this is
true it helps to explain the relative independence of shoot growth from ex-
ternal conditions during that season. Presumably there must be species
differences in the amount of food accumulated and the rate at which this is
digested and translocated in the spring to the growing stem tips. This
might explain the difference in seasonal distribution of growth existing, for
example, between white ash and yellow poplar or between the northern and
southern pines. It seems possible that trees with long growing seasons
might not only utilize reserve food, but later in the same season they might
also utilize the products of current photosynthesis in shoot growth as well as
in cambial activity, thus prolonging their growing season beyond that of
species which depend chiefly or entirely on reserve food. There appears to
be no definite relation between length of growing season and amount of shoot
growth. White ash has a short period of growth and yellow poplar a long
period, but both make about the same amount of growth. While loblolly
pine seedlings make considerably more growth than slash and shortleaf pine,
their growing season is only a few days longer and this could not explain the
difference in amount of growth.

Another feature of the growing season of many tree species to which
attention should be called is the relatively small portion of the frost-free

season in which most of the shoot growth occurs. This was emphasized by ILLICK (7) who reported that at Mont Alto, Pennsylvania, 90 per cent. of all shoot growth occurs in 40 days, or about 25 per cent. of the frost-free season, and most species cease shoot growth in June. Ten species growing at Durham, North Carolina, had an average growing season of 160 days, or 80 per cent. of the frost-free season and made 90 per cent. of their growth in 100 days, or 50 per cent. of the frost-free period. The five hardwood species had an average growing season of 140 days or only 60 per cent. of the frost-free season and made over half of the season's growth in about 30 per cent. of the frost-free season. The average growing season of the species measured by KIENHOLZ in Connecticut and New Hampshire was 65 to 70 per cent. of the frost-free season; those trees, like the trees in North Carolina, averaged 90 per cent. of their growth in about 50 per cent. of the frost-free period. Apparently very few tree species in eastern North America have a growing season equal to the frost-free season, the only such species known to the writer being loblolly, slash, and shortleaf pine observed in this study, and eastern larch observed by KIENHOLZ (11). Even these species ceased growth two or three weeks before the average date of the first killing frost. It was possible for them to have a growing season equal in length to the frost-free season only because they had started growth before the average date of the last killing frost in the spring. Not only do most trees cease shoot growth several weeks or even months before the first frosts of autumn, but they also resume growth in the spring before the danger of frost is over. This is not only true in North Carolina but also in Pennsylvania, New England, and probably all over the northern hemisphere. BALDWIN (1) mentions severe frost injury in New England; DAY and PEACE (5) report that it is common in England and it is mentioned by various continental writers (3).

This puzzling situation constitutes one of the most interesting problems in tree physiology. Why should most trees resume shoot growth in the spring before the danger of frost is past, then cease growth in midsummer or at least weeks before the first killing frost of autumn? Cessation of growth in midsummer does not have any obvious survival value to the species, but would rather serve to decrease its ability to compete with species having longer growing seasons. Of course, if shoot growth continued until the first frost the succulent tips might be killed, but there is no reason to suppose that occasional injury of this sort would be any more serious than the injury caused by spring frosts. The writer has caused yellow poplar, loblolly, and slash pine to grow until frost by exposing them to a long photoperiod and although some stem tips were killed the growth of the trees the following season was not measurably less than that of uninjured trees.

Well over half a century ago SACHS (19) observed that growth frequently ceased while temperature and other conditions favored vegetative activity and was resumed under conditions which appeared to be far less favorable. He stated that while the dormant period occurred at a definite season and therefore appeared to be dependent on temperature and moisture, it really in his opinion, depended chiefly on changes occurring within the plant. It

is clear that while increasing soil and air temperatures are important factors in determining the time of resumption of growth in the spring, decreasing soil and air temperatures certainly do not determine the time of cessation of growth, because growth of most species ceases before air and soil temperature begins to decrease. Severe droughts might prematurely stop growth, but ordinary fluctuations in rainfall do not, nor can they explain why some species cease growth long before others. KLEBS (12) in one of the earlier discussions of the periodicity of tree growth suggested that temporary depletion of minerals might cause the cessation of growth by tropical trees, but this would scarcely explain the difference in behavior of different species in the same habitat. He also suggested that length of day may be an important factor in determining the length of the growing season, although he seems to have been thinking in terms of increased photosynthesis rather than photoperiod. JESTER and KRAMER (8) observed that seedlings of a number of species of trees, both in the greenhouse and out-of-doors, grew much later in the season, when given a photoperiod as long as that of midsummer by use of supplementary electric lights, than did controls exposed to the normally decreasing photoperiod of late summer and autumn.

The species used in the present study were also planted in a nearby plot illuminated each evening after the days began to shorten so that they always received a photoperiod as long as that of midsummer. Yellow poplar, loblolly pine and slash pine showed definite responses, growing about three weeks later under the lights than without them. Yellow poplar continued to form new leaves until frost and slash pine showed considerable winter killing of stem tips, probably because the new growth did not have time to harden before the first frost. There is no doubt that the length of growing season of many species can be affected by the photoperiod under experimental conditions, but the growing season of some species is not affected. It is probable that photoperiod affects the growing season of some species in nature, but there is no clear evidence that it is the only or even the major factor controlling the time at which trees cease growth.

Obviously more information must be collected before we can explain the differences in growth habits of trees. While considerable data are available on the relation between growth and environmental conditions, more might profitably be collected. It would be particularly valuable to have more data on the response of seedlings to variations in environmental factors under controlled or at least partly controlled conditions of soil and air temperature, soil moisture, and photoperiod. Much more information concerning the physiology of species with long and short growing seasons is also needed, with special reference to food accumulation and its subsequent utilization in growth.

Summary

Eleven species of tree seedlings were grown together in the same plot for three years at Durham, North Carolina, and their growth measured at intervals through the growing season.

Of the five hardwoods, white ash and yellow poplar grew most rapidly, making over twice as much growth as red and white oak, and black walnut. Loblolly pine grew more rapidly than any other of the six conifers. Short-leaf and slash pine made about two-thirds the growth of loblolly, but the northern species, red and white pine and balsam fir, made only one-sixth the growth made by loblolly pine.

There were also considerable differences between species with respect to length of growing season and time of maximum growth. All species resumed growth in late March or early April. White ash, eastern red oak, white oak, and black walnut made about 50 per cent. of their growth by May 1 and ceased growth in July or early August after growing seasons of 130 to 135 days. Yellow poplar had no peak month, but grew about the same amount every month from April through July and finally ceased growth in September after a growing season of 160 days.

Loblolly, shortleaf, and slash pine behaved alike, starting growth in late March, making 15 to 20 per cent. of their total growth each month from April through August, and finally ceasing growth in October. Red and white pine, which are characteristic of more northern latitudes and higher altitudes, resumed growth very little sooner than the southern species but made 65 per cent. of their growth by May first, 90 per cent. by June first, and ceased growth in July or August after a growing season of 120 to 145 days, compared to 200 days or more for the southern species.

Trees growing in North Carolina uniformly had longer growing seasons than the same species in New England, but most of the growth occurred at the beginning of the season and the trees did not benefit by the longer grow-ing season. Red and white pine and balsam fir made less growth and had a higher mortality than would have been expected in New England. The seasonal course of growth of a species was the same in North Carolina as in New England. The shape of the growth curve is apparently determined largely by internal factors rather than environmental factors.

Most tree species for which data are available behave alike, both in New England and North Carolina, in that they all resume growth in the spring before danger of frost is past but most of them cease growth in mid or late summer, long before the first frost of autumn. As a result many species use only one-half to three-fourths of the frost-free season.

While decreasing photoperiod probably checks the growth of some species it is not the only factor involved. Much more research will be necessary to give us a satisfactory understanding of the complex of factors controlling the course of growth and length of the growng season.

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LITERATURE CITED

1. BALDWIN, H. I. The period of height growth in some northeastern conifers. Ecology **12**: 665–689. 1931.

2. BURGER, H. Untersuchungen über das Höhenwachstum verschiedener Holzarten. Mitt. schweiz. Centralanstalt först. Versuchswesen **14**: 1–158. 1926.

3. BÜSGEN, M., and MÜNCH, E. The structure and life of forest trees. Eng. transl. New York. 1931.

4. COOK, D. B. Five seasons' growth of conifers. Ecology **22**: 285–296. 1941.

5. DAY, W. R., and PEACE, T. R. The experimental production and diagnosis of frost injury on forest trees. Oxford Forestry Mem. 16. 1934.

6. FRIESNER, R. C. Vertical growth in four species of pines in Indiana. Butler Univ. Bot. Stud. **5**: 145–159. 1942.

7. ILLICK, J. S. When trees grow. Amer. Forestry **25**: 1386–1390. 1919.

8. JESTER, J. R., and KRAMER, P. J. The effect of length of day on the height growth of certain forest tree seedlings. Jour. Forestry **37**: 796–803. 1939.

9. JOHNSTON, J. P. Height-growth periods of oak and pine reproduction in the Missouri Ozarks. Jour. Forestry **39**: 67–68. 1941.

10. KIENHOLZ, R. Leader, needle, cambial and root growth of certain conifers and their interrelations. Bot. Gaz. **96**: 73–92. 1934.

11. —————. Seasonal course of height growth in some hardwoods in Connecticut. Ecology **22**: 249–258. 1941.

12. KLEBS, G. Über das Treiben der einheimischen Baüme speziell der Buch. Reviewed Plant World 18: 19. 1915. Forest Quart. **14**: 83. 1916.

13. —————. Über Wachstum und Ruhe tropischer Baumarten. Jahrb. wiss. Bot. **56**: 734–792. 1915.

14. KORSTIAN, C. F. Private communication. 1939.

15. —————, and MAUGHAN, W. The Duke forest. Duke University School of Forestry Bull. 1.

16. LOOMIS, W. E. Translocation and growth balance in woody plants. Ann. Bot. **49**: 247–272. 1935.

17. REED, J. F. Root and shoot growth of shortleaf and loblolly pines in relation to certain environmental conditions. Duke University School of Forestry Bull. 4. 1939.

18. REED, H. S., and MacDOUGAL, D. T. Periodicity in the growth of the orange tree. Growth **1**: 371–373. 1937.

19. SACHS, J. Lectures on the physiology of plants. Eng. transl. Oxford. 1887.

20. TRYON, H. H., and FINN, R. F. Notes on the terminal growth of coniferous plantations in the Hudson highlands. Black Rock Forest Papers **1**: 54–56. 1937.

TRANSPIRATION RATES OF SOME FOREST TREE SPECIES DURING THE DORMANT SEASON

THEODORE T. KOZLOWSKI

(WITH TWO FIGURES)

This paper reports the results of a comparison of the winter transpiration rates of several deciduous and evergreen tree species. The species were: white oak (*Quercus alba* L.), yellow poplar (*Liriodendron tulipifera* L.), sugar maple (*Acer saccharum* Marsh.), cherry laurel (*Prunus laurocerasus* L.), eastern white pine (*Pinus strobus* L.), and loblolly pine (*Pinus taeda* L.).

Methods

Six individuals of each species, each about three years old, were transplanted from clay pots to cylindrical metal buckets of five-quart capacity. In transplanting the seedlings the entire core of soil containing the root system was transferred intact to reduce disturbance of the roots to a minimum. The soil used was a sandy loam with a field capacity of 23.2 per cent. After transplanting, the specimens were placed outdoors for a week prior to beginning experimental work. To prevent catchment of rain water and evaporation from the soil surface the buckets were sealed with two layers of oil cloth which sloped from the stems to the rims of the metal buckets at an angle of approximately 55 degrees. The oil cloth was heavily coated with a sealing compound consisting of paraffin, tallow, beeswax, and rosin. A glass tube, closed by a cork stopper, was inserted through each cover to permit the addition of water.

The experiment was performed on a flat roof where there was a minimum of shading by nearby trees and buildings. The specimens were arranged in six randomized blocks with each block having six plants representing each of the six species. The six blocks were located in a bed containing excelsior packing to prevent excessive changes in soil temperature as well as unnatural freezing. The blocks were so arranged that, although the afternoon shade affected the blocks differently, the plants within each block were shaded to the same extent. This arrangement was made to eliminate within-block variations due to shading differences.

The soil in the buckets at the beginning of each experimental period was at the previously determined field capacity, and the equivalent of the transpired water was replaced immediately after each experimental period. Weather data were obtained with a Friez hygrothermograph. Evaporation rates were obtained with standard Livingston atmometers. Total wind velocity for each experimental period was obtained with a cumulative recording anemometer. The gravimetric method of measuring transpiration was employed using a balance with a sensitivity of one gram. Loss in

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weight during the experimental period was assumed to represent the weight of the water lost in transpiration.

The leaf areas of the broad-leaved evergreen species (*Prunus laurocerasus*) were determined by the photoelectric cell method modified and described by KRAMER (6). The stem surface areas of the deciduous species were obtained by taking diameter measurements at one inch intervals with a microcaliper along the stems and branches to establish the mean diameter. The diameter and total lengths of the stem and branches were readily converted to surface areas. The transpiring surface area (stomate bearing surfaces) of loblolly pine and eastern white pine were determined by the method described by KOZLOWSKI and SCHUMACHER (5). It was assumed that the transpiration rate from non-stomated leaf surfaces was negligible and these surfaces were disregarded in the calculations.

Results and discussion

Figure 1 indicates weekly losses for the evergreen species in grams of water transpired per square decimeter of stomate-bearing leaf surface and
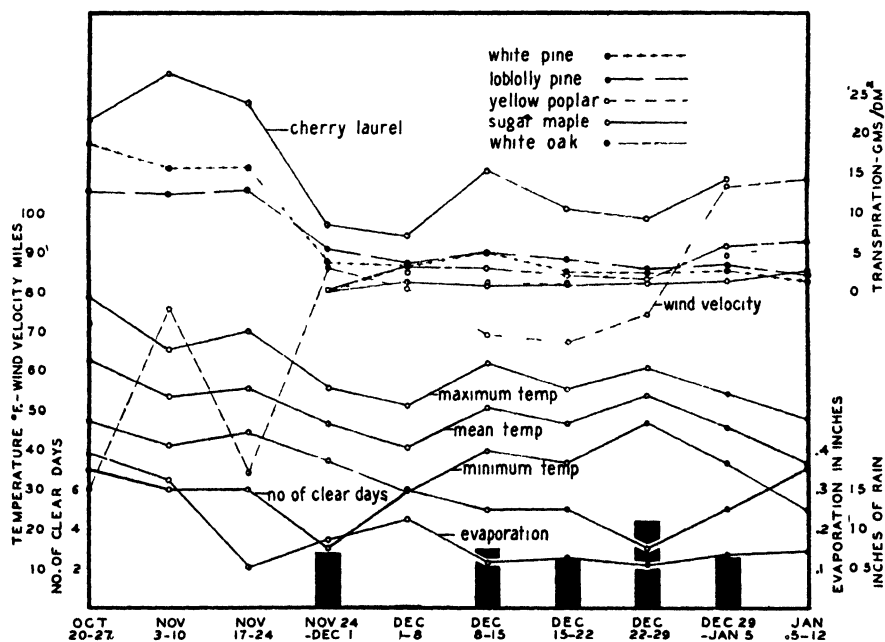
FIG. 1. Relationship between total weekly transpiration rates of six species during the dormant season and certain atmospheric variables. Data for six individuals of each species in Durham, North Carolina, from October, 1940, to January, 1941. Transpiration given in grams of water lost per week per square decimeter of transpiring area. Black vertical bars represent total rainfall in inches.

per square decimeter of exposed stem area for the deciduous species. Weekly losses for the evergreen species in grams of water transpired per gram of oven-dry weight of foliage are presented in table I. Data of figure 1 agree generally with the findings of WEAVER and MOGENSON (10) in that

they indicate no great differences between the transpiration rates of conifer-
ous and deciduous species during the dormant season on a unit area basis.
WEAVER and MOGENSON worked with broad-leaved and coniferous species
under uniform conditions of soil type and texture, soil temperature, and
identical aerial environment; they reported that autumn transpiration losses
from conifers were just as great as, or even greater than, those from broad-
leaved species.   They found that winter losses by transpiration from coni-
fers were only 1/55 to 1/251 as great as those in autumn.   Increased losses
of broad-leaved trees in spring occasioned by foliation were reported to be
in proportion to the leaf areas exposed and were clearly controlled by
weather conditions; although essentially, they were comparable to losses in
conifers.   They found that winter transpiration rates from conifers were
little greater than those from defoliated stems of broad-leaved trees.   The
winter rates of these investigators were much lower than those reported
herein, resulting in smaller seasonal differences than reported in their study.

Data of table I are in general harmony with findings of KUSANO (8) who
presented quantitative data on transpiration of evergreen species indigenous
to Japan.   He found that in winter non-coniferous evergreen trees trans-
pired an average quantity of at least 0.48 grams per square decimeter of
leaf surface per day or 16.58 grams per 100 grams of fresh weight in foliage
trees, while conifers transpired 8.18 grams per day.   He also presented data
which indicated that the time of minimum transpiration agreed with that
of minimum temperature, which occurred at the end of January.   He re-
ported that on the average the amount of water lost in transpiration by
broad-leaved evergreen trees was one and one-half or two times greater than
that lost by conifers if the amount were reduced either to the fresh weight
or to the dry weight of the transpiring part of the plant.   It will be noted
in table I that, during December and January, transpiration rates of cherry
laurel when reduced to the oven-dry weight of the transpiring part of the
plant were consistently higher than those of the pines.   The amount of
water lost by cherry laurel varied from 1.26 to 1.87 times as much as that
of loblolly pine for weekly periods during December and January.   During
the same period, rates of cherry laurel varied from 1.80 to 3.11 times as
great as those of white pine.   During warmer seasons these relations did not
seem to hold.   Rates of both white pine and loblolly pine were somewhat
higher than those of cherry laurel in late October on a dry weight of foliage
basis.   With lowering of average temperature, rates of cherry laurel were
proportionally increased over those of the pines until the differences reported
were recorded (table I).

Using transpiring surface area as a basis, the absolute rates of cherry
laurel were consistently higher than those reported by KUSANO for broad-
leaved evergreens.   The average daily loss for cherry laurel was from 1.00
gram to 2.16 grams of water per square decimeter of leaf surface or the
minimal loss was approximately twice that reported by KUSANO.

An analysis of variance as described by FISHER (4) was made for dor-

mant season rates on the basis of transpiring surface area from data for the six week period of November 24, 1940, to January 5, 1941; it was not until the former date that the deciduous species were completely defoliated. The analysis indicates that when all species are considered, highly significant effects on transpiration rates were due to species, time, and the interaction of time and species. The differences due to species are primarily due to the rates of cherry laurel which were higher than those of any other species when put on a leaf-area basis. The treatment or species sum of squares from the analysis was further broken up into five independent comparisons among the six species. A comparison between the evergreen species, including two pines and one broad-leaved species, against the deciduous species indicates significance at the one per cent. level. The indication is that

TABLE I

TRANSPIRATION OF EVERGREEN SPECIES IN GRAMS PER GRAM OF OVEN-DRY WEIGHT OF LEAVES
FOR SEVEN-DAY PERIODS. DATA OF OCT. 20, 1940, TO JAN. 12, 1941.
AVERAGE VALUES FOR SIX PLANTS OF EACH SPECIES

	LOBLOLLY PINE	WHITE PINE	CHERRY LAUREL
	gm.	gm.	gm.
Oct. 20 to 27	24.382	26.369	18.782
Nov. 3 to 10	23.983	23.520	23.497
Nov. 17 to 24	24.793	23.614	20.548
Nov. 24 to Dec. 1	10.348	7.324	7.363
Dec. 1 to 8	6.552	5.432	6.070
Dec. 8 to 15	9.643	7.312	13.136
Dec. 15 to 22	7.716	4.053	9.019
Dec. 22 to 29	5.523	3.440	8.029
Dec. 29 to Jan. 5	6.588	3.969	12.352
Jan. 5 to 12	3.846	1.783	

the observed differences would be due to chance not more than one time in one hundred. The magnitude of the real difference in rates between the pines and the cherry laurel against the deciduous species is indicated by a derived variance ratio of 46.68 whereas a ratio of only 7.77 would indicate significance at the one per cent. level for the degrees of freedom involved. The great difference between rates of the two pines and those of cherry laurel is indicated by a variance ratio of 56.21 for this independent comparison. The remaining independent comparisons were of white oak against yellow poplar, white oak against yellow poplar and maple, and yellow poplar against maple. These three comparisons were not statistically significant.

Soil temperature effects

The low winter transpiration rate of the conifers is partly caused by atmospheric factors reducing transpiration and partly by low soil temperature decreasing absorption. The importance of low soil temperature in decreasing transpiration was determined by growing plants in the greenhouse where the soil could be cooled while the air surrounding the tops was

maintained at normal greenhouse temperatures. A temperature control apparatus, consisting of four metal tanks, each equipped with a thermoregulator, heating coils, and a refrigerating unit was used. With this equipment the water in each tank could be maintained at any constant temperature from 0° to 40° C.

Seedlings were sealed in buckets, as in the experiments described above, and were randomized into four groups of 6 plants each. Three of the 6 plants in each group were loblolly pines and three were eastern white pines. Each water bath was adjusted to a constant temperature of 30° C. One group of buckets consisting of three white pines and three loblolly pines was placed in each tank so that each bucket was immersed. The position of the bucket within the tank was selected at random. The plants were kept in the water bath at 30° C. for three days in order to overcome the effects of the previous soil temperature. At the end of this preliminary three-day treatment each bucket was weighed and the moisture content of the soil within it brought back to the field capacity. Each plant was replaced in the position it had occupied within the tank and transpirational losses for a 90-hour period were determined gravimetrically. One tank was then maintained at 30° C. while the other three were adjusted to 0°, 5°, and 17° C. Three-day preliminary runs were made to overcome effects of previous soil temperatures before losses for 90-hour periods at the 4 temperatures were determined. After rerandomizing buckets between and within tanks, the entire second run was repeated. This yielded results essentially similar to those of the first run.

The transpiration rates of white pine and loblolly pine under the effects of varying soil temperatures are expressed as percentages of the rate at 30° C. in figure 2. Since one tank was maintained at 30° C. for both runs, it was assumed that, if atmospheric conditions were similar during both runs, transpiration rates of both runs would be of similar order, or the ratio in percentage would be 100. Since atmospheric conditions were not identical in successive runs some departure from the 100 per cent. ratio was recorded and a correction was necessarily applied to the rates of the plants in soils maintained at the lower temperatures.

In figure 2 it will be noted that for both species the maximum transpiration rate occurred at the highest control temperature, 30° C., and that a reduction in soil temperature reduced the rate of transpiration of both species. It is of interest to note the varying trends of the transpiration curves for these two species. For eastern white pine the average rate in a soil at 1° C. was approximately 21 per cent. of that of the control group; at 5° C. it was approximately 70 per cent. of that of the control group; and at 17° C. it was approximately 76 per cent. of that of the control group. For loblolly pine, however, the average rate at 1° C. was approximately 23 per cent. of that of the control group; at 5° C. it was approximately 51 per cent. of that of the control group; and at 17° C. it was approximately 74 per cent. of that of the control group. The results obtained are quite similar

to those of CLEMENTS and MARTIN (2) who investigated effects of soil temperature on the transpiration rate of *Helianthus annuus,* although they found a lesser reduction in transpiration from 30° to 15° C. They reported
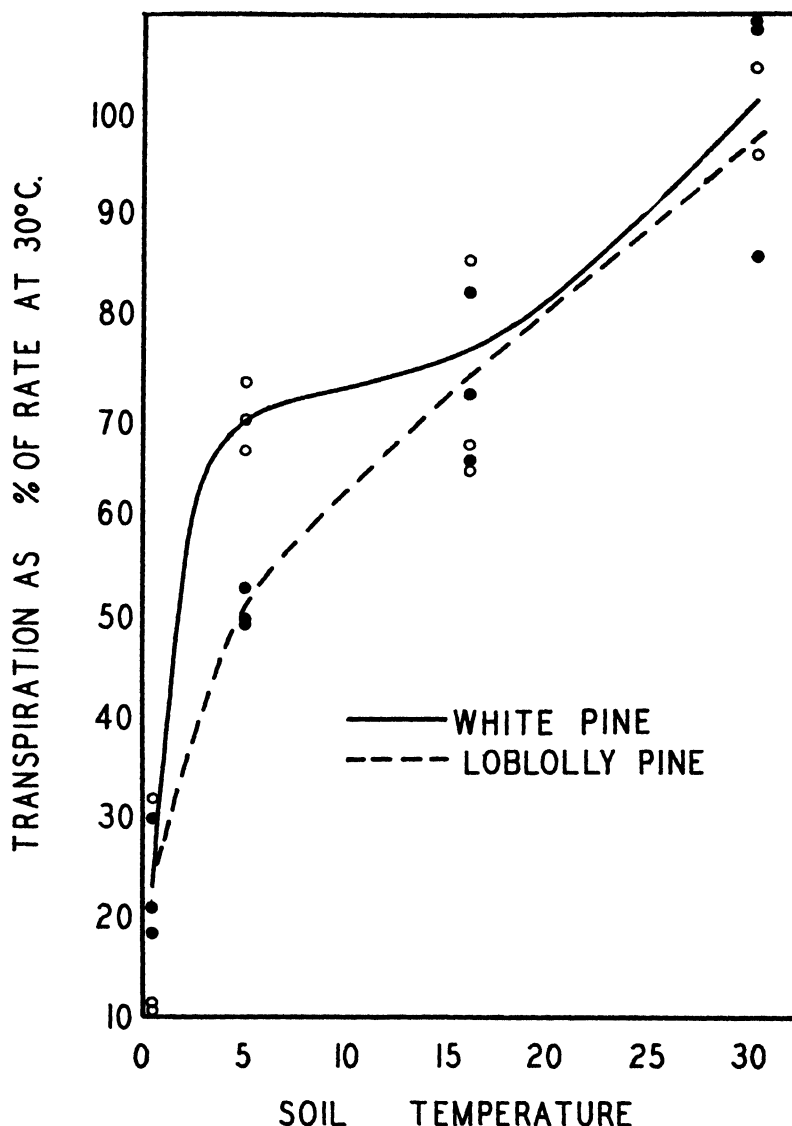
FIG. 2. Effects of soil temperature on transpiration rates of eastern white pine and loblolly pine. Observations for eastern white pine recorded as circles. Temperature in degrees Centigrade.

only a slight decrease in this range, but a very rapid decrease below 13° C., the reduction being one-half at 3° C. Plants began to wilt at about 4.5° C. and were completely wilted at 1° C. Warming the soil caused rapid recovery. ARNDT (1) reported that cotton in solution cultures wilted when their

temperature had been lowered to between 10° and 18° C.; in soil cultures, with ample water supply, the highest soil temperature at which wilting occurred was generally somewhat higher, between 17° and 20° C. DÖRING (3) used potometers and plants with roots submerged in water in studying effects of temperatures in the range of 20° to 0° C. on transpiration. He reported that decreases in transpiration rate due to lowered soil temperatures varied with species; the rate in some being decreased 70 to 80 per cent. while in other species it was not decreased at all. MICHAELIS (9) has suggested that high rates of transpiration when the soil is frozen and absorption is too slow to replace water loss may be an important factor in determining timber line in mountainous country.

Effects of low soil temperatures on water absorption and transpiration are operative in several ways which probably are interacting. KRAMER (7) cites several papers in summing up the following effects of low temperature on plant reactions: A decreased temperature decreases the rate of movement of water from the soil to the absorbing root surfaces. Low temperatures exert an effect in retarding root elongation. Since the continual extension of root tips into contact with the water films surrounding hitherto untouched soil particles is important in making soil moisture available a decrease or cessation of root elongation probably decreases the rate of absorption. This would be most important in soils with a moisture content below the field capacity, a condition which commonly exists in the field. Cell permeability is decreased as temperature is lowered while the viscosity of protoplasm and of colloidal gels in the cell walls is much higher at lower temperatures. The viscosity of water increases as temperature decreases and is twice as high at 0° as at 25° C. This results in a decreased rate of movement from soil to roots and through the root cells. Physiological activity, notably respiration rate, is markedly decreased by low temperatures.

Although absorption may be seriously affected by changes in the viscosity of the water itself, figure 2 indicates that colloidal properties of the protoplasm are also important in controlling absorption. The shape of the curve for loblolly pine more closely approaches that for the reciprocal of the viscosity of water than does the shape of the curve for eastern white pine. This suggests that low soil temperatures may cause a greater decrease in the permeability of the roots of loblolly pine to water than of those of eastern white pine. At low soil temperatures above freezing the greater reduction of absorption due to inherent protoplasmic qualities of loblolly pine might partly explain its inability to survive in colder regions.

Summary

An investigation was made of the absolute transpiration rates of loblolly pine, eastern white pine, cherry laurel, white oak, yellow poplar, and sugar maple during the dormant season of 1940–1941 in Durham, North Carolina. Absolute rates are reported for the 6 species in grams of water transpired per square decimeter of transpiring surface and also for the evergreen

species in grams of water transpired per gram of oven-dry weight of foliage. The effects of soil temperature on transpiration rates of loblolly pine and eastern white pine were studied.

The results agree generally with those of WEAVER and MOGENSON (10) in that no great difference was observed in foliar transpiration of conifers and stem transpiration of deciduous species on a unit area basis. Smaller differences between autumn and winter rates were found than those reported by these investigators. This was probably because their winter temperatures were much lower than those in the present experiments.

The October–November average rate for loblolly pine and cherry laurel was more than twice the average maximum for December and January; for white pine the October–November average rate was more than three times the December–January maximum.

Independent comparisons of the evergreen versus deciduous species and of pines versus cherry laurel indicated highly significant differences on a unit area basis. During December and January the weekly transpiration rates of cherry laurel were approximately from 2 to 4 times as great as those of either of the pines. Comparisons of rates of loblolly pine versus eastern white pine, white oak versus yellow poplar and sugar maple, and yellow poplar versus sugar maple indicated no real statistical differences in transpiration of these species during the dormant season.

A decrease in soil temperature was found to decrease the transpiration rates of loblolly pine and eastern white pine. This is in general agreement with results obtained by other investigations with herbaceous materials. Transpiration of loblolly pine was reduced more than that of eastern white pine over the temperature range between 17° and 0° C. The difference in behavior of the two species is probably caused by differences in inherent protoplasmic qualities. The greater reduction in absorption by loblolly pine in cold soil might be a factor in its inability to survive in colder regions.

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LITERATURE CITED

1. ARNDT, C. H. Water absorption in the cotton plants as affected by soil and water temperature. Plant Physiol. **12**: 703–720. 1937.

2. CLEMENTS, F. E., and MARTIN, E. V. Effect of soil temperature on transpiration in Helianthus annuus. Plant Physiol. **9**: 619–630. 1934.

3. DÖRING, B. Die Temperaturabhängigkeit der Wasseraufnahme und ihre ökologische Bedeutung. Zeitschr. Bot. **28**: 305–383. 1935.

4. FISHER, R. A.   Statistical methods for research workers.   Oliver and Boyd.   London.   1936.

5. KOZLOWSKI, T. T., and SCHUMACHER, F. X.   Estimation of stomated foliar surface of pines.   Plant Physiol. **18**: 122–127.   1943.

6. KRAMER, P. J.   An improved photoelectric apparatus for measuring leaf areas.   Amer. Jour. Bot. **24**: 375–376.   1937.

7. ——————.   Root resistance as a cause of decreased water absorption by plants at low temperatures.   Plant Physiol. **15**: 63–79. 1940.

8. KUSANO, S.   Transpiration of evergreen trees in winter.   Imp. Univ. Tokyo Jour. Col. Sci. **15**: 313–366.   1901.

9. MICHAELIS, P.   Ökologische Studien in der alpinen Baumgrenze.   IV. Zur Kenntnis der winterlichen Wasserhaushaltes.   Jahrb. wiss. Bot. **80**: 169–247.   1934.

10. WEAVER, J. E., and MOGENSON, A.   Relative transpiration of coniferous and broad-leaved trees in autumn and winter.   Bot. Gaz. **68**: 393–424.   1919.

A NOTE ON THE GROWTH BEHAVIOR OF COTTON BOLLS[1]

DONALD B. ANDERSON AND THOMAS KERR

(WITH SEVEN FIGURES)

The wall of every cotton fiber is made up of two component parts: (a) the primary wall and (b) the secondary wall (1). The primary wall, an extremely thin layer deposited by the elongating epidermal cell, is formed during a period of about sixteen days following the opening of the cotton flower. During this period the fiber reaches its full length, but the wall remains extremely thin and delicate. About the time that the fiber ceases to elongate the second phase of its development, that of wall thickening, begins. Wall thickening occurs continuously and simultaneously over the entire inner surface of the fiber for the succeeding twenty or more days. The wall deposited during this second phase of development is the secondary wall of the fiber cell. It is this secondary wall that is responsible for the tensile strength of the cotton fiber.

The tensile strength of cotton fiber is influenced by many factors, both environmental and genetic. In the course of an investigation of these factors, it was noticed that the tensile strength of the fibers appeared to increase whenever the plants were subjected to a deficiency of water during the period of secondary wall formation. This observation suggested that measurements of water stress within the plant at the time of secondary wall deposition might serve as an index to the tensile strength of the mature fiber. Efforts were made therefore to obtain some record of the variations in water stress within the plant during the period of fiber growth.

The most obvious indication of the severity of water stress in plant cells is the magnitude of cell turgor. Variations in the turgor of the cells composing the young seeds and bolls should be reflected by corresponding changes in the volume and in the diffusion pressure deficits of the cells of these organs. Measurements of these quantities were undertaken and this paper is a report of the results of studies of the changes in the volume of growing cotton bolls during the summers of 1940, '41, and '42.

Methods

Continuous records of the diurnal and seasonal changes in the diameters of cotton bolls were obtained with auxometers designed for this purpose. A young cotton boll was pressed lightly against a vertical glass plate by a movable lever arm in such a way that any change in the diameter of the boll would induce a movement of the lever arm. The slight movements of the lever arm were multiplied by a series of levers so that any changes in

Fɪɢ. 1. The auxometer. A detached boll has been placed between the lever arm and the glass plate—(right end of the apparatus)—to illustrate the position that the boll occupies when the machine is in operation.

boll diameter were increased about 40 times at the point where they were recorded, in ink, upon a rotating drum. The apparatus (fig. 1) was constructed largely of wood with a pen arm of glass tubing supported upon bronze knife-edge bearings by a short brass axle. The wood was protected from expansion or contraction resulting from the absorption or loss of water by means of aluminum foil. Each piece of wood in the instrument was covered, before assembly, with five layers of aluminum foil cemented together with white lead and linseed oil (5). This method of protecting the
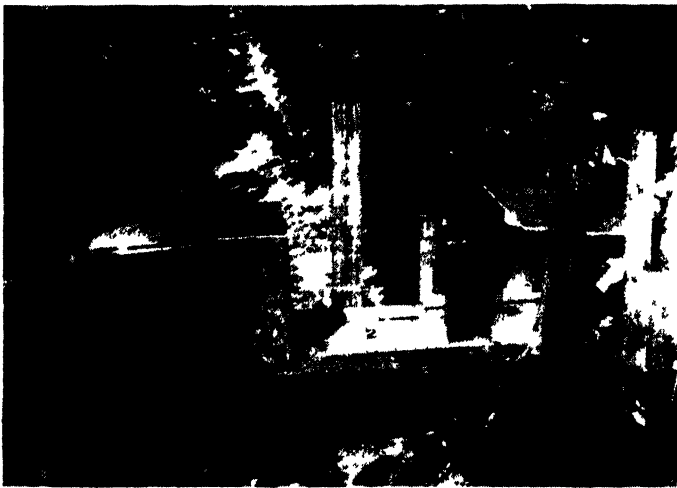
Fɪɢ. 2. The auxometer mounted in the field. The rotating drum is covered with a metal housing with a glass window to permit observation. The boll is shaded from the direct sunlight by means of a small square of plywood which is visible at the right.

wood was entirely successful so that in a test run, with a quartz pebble substituted for a cotton boll, a straight line was recorded on the drum during a very warm day followed by a severe thunder storm. The rotating drums used were those manufactured by Julien P. Friez and Sons for their recording thermometers. The pen recording mechanism was similar to that employed on the Friez instrument just mentioned. Records were obtained every day upon thermograph charts, the change of charts being made about sunset in 1940–1941, and at 8:30 A.M. (war time) in 1942.

The auxometer was mounted upon a 3-ft. length of a 2″ × 6″ plank which in turn was supported upon stakes driven solidly into the ground (fig. 2). The branch bearing the boll was tied firmly in place with raffia, and the main stem of the plant was likewise securely fastened with raffia to a substantial stake. The bolls were held in place so firmly that the position of the boll in the instrument was not altered by winds prevalent during the summer.

Since it was important for our purpose to determine the diurnal and seasonal variations in the diameter of bolls growing under field conditions, the instruments were mounted in fields of the North Carolina Agricultural Experiment Station Farm at Raleigh. Five auxometers were operated simultaneously in a single experimental plot so that records were obtained of the growth behavior of bolls of different ages under the same environmental conditions. Although measurements of diameter changes in bolls of several strains of upland cotton (Rowden, Half and Half, Coker 100, Cook, and D & P L) were obtained during the three summers' work no significant differences in behavior were observed in any of the varieties studied.

It is recognized that changes in the *diameter* of a globose fruit like the cotton boll do not represent very adequately the actual magnitude of the variations in *volume* since the relation of the volume of a sphere to the radius is expressed by the formula $4/3 \pi R^3$. The total volume changes in the boll are, of course, much greater than those indicated by the growth curves presented in this paper. The records of the diameter changes as recorded by the auxometer do indicate, however, the general pattern of growth behavior very well, and it has seemed better for various reasons to use the auxometer records directly rather than to convert these into volume changes. All of the growth curves presented in this paper are, therefore, variations in the diameter of the bolls as recorded automatically by the instrument.

Results

It soon became apparent that the response of young, enlarging bolls to the internal water stress of the parent plant was entirely unlike that of full-sized bolls. So long as the boll was growing in size, *i.e.*, during the period of primary wall formation in the fiber cells, the increase in diameter was continuous during both the day and the night. Enlargement during the day exceeded that which occurred during the night, indicating that the higher temperatures of the day had a greater influence upon growth than the more favorable water relations existing during the night. This rapid enlargement

of the young bolls (7 to 12 days old) during the day continued to occur during periods of severe drought when the leaves of the cotton plants were usually wilted by 10 A.M. and even when the leaf blades were hanging limply from the petioles in the afternoon (fig. 3). As the bolls approached full size (13 to 15 days), however, the rate of enlargement was noticeably checked by severe wilting of the parent plant and sometimes slight shrinkage of the bolls occurred.
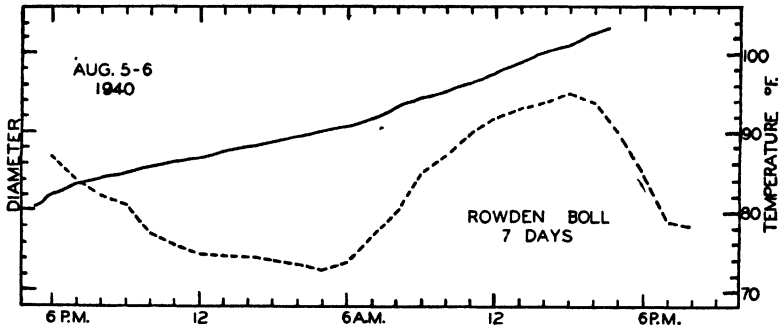
FIG. 3. Diurnal variations in the diameter of a young cotton boll during a 24-hour period in August (solid line). Temperature variations are shown by the broken line. The parent plant exhibited marked wilting before noon but the water stress within the plant failed to check enlargement.

As soon as the bolls reached their full size, *i.e.*, as soon as the secondary walls began to form in the fibers, the pattern of diurnal variations in diameter was completely altered. The bolls decreased in size during the warm part of the day when wilting of the parent plant occurred and regained their full size during the night (fig. 4). When soil moisture was near field
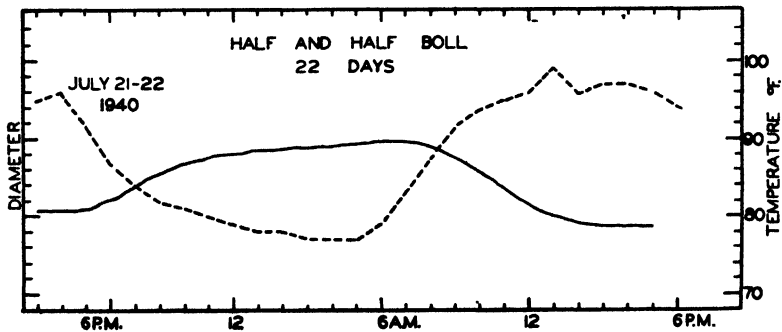
FIG. 4. Diurnal variations in the diameter of a full-sized boll during a warm day and night in July (solid line). Temperature variations are shown with the broken line. Shrinkage begins in the morning about the time that wilting is visible in the leaves.

capacity some shrinkage in size of the bolls occurred before the first evidence of wilting became visible in the leaves. As soil moisture decreased, shrinkage increased in amount during the day and recovery was slower during the night. Under these conditions shrinkage did not occur in the bolls until after the leaves were visibly wilted. The amount of shrinkage was in general directly proportional to the degree of the wilting visible in the leaves.
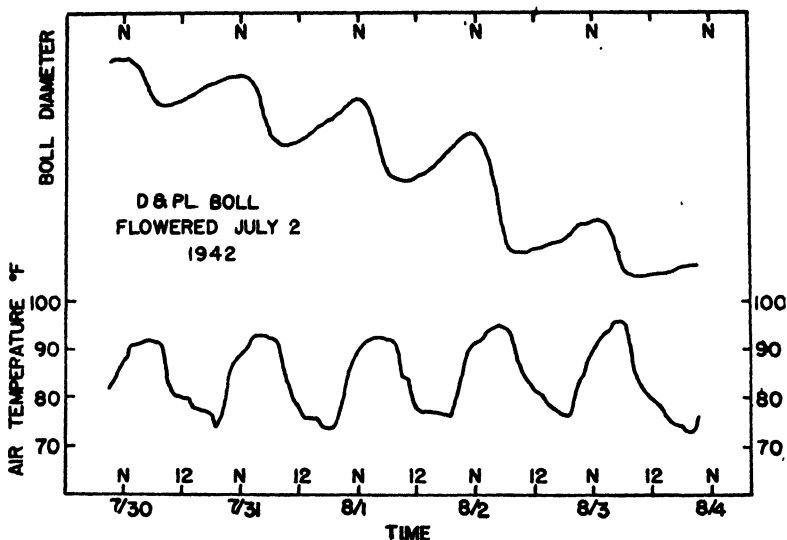
FIG. 5. Variations in the diameter of a full-sized boll during a period of severe drought. The plant was approaching permanent wilting on Aug. 4.

In the summer of 1942, drought was so severe that the cotton plants closely approached permanent wilting. Under such severe water stress, the full sized bolls continued to show shrinkage during the day but the decrease in diameter did not begin until early in the afternoon although the leaves were severely wilted by 10 A.M. They did not recover completely at night so that the bolls became smaller day after day (fig. 5). When permanent wilting occurred there was practically no recovery of bolls during the night.

In figure 6 the growth behavior of a full sized boll is shown in the six
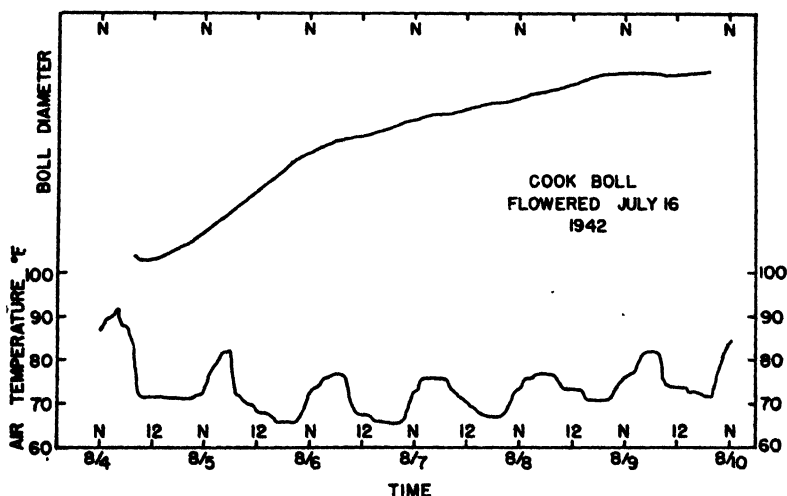
FIG. 6. Variations in the diameter of a full-sized boll as it recovered from severe drought.

days immediately following the period of permanent wilting. During this six-day interval the air temperatures were relatively low, rains were frequent and the sky was overcast most of the time. The boll increased in size continuously but not uniformly until the plant attained its maximum turgor by which time the boll exhibited no further increase in diameter. A similar pattern of growth behavior was shown by the boll in each of the five auxometers. When the plants are turgid an hour or two of sunshine in the middle of the day is sufficient to induce some shrinkage in the full sized bolls. This behavior stands in marked contrast to the severe wilting that is necessary to induce shrinkage when soil moisture is seriously deficient.

Figure 7 represents a continuous record of the growth of a single cotton boll for a period starting 11 days after the opening of the flower and ending 16 days later. The marked contrast between the diurnal variations in volume
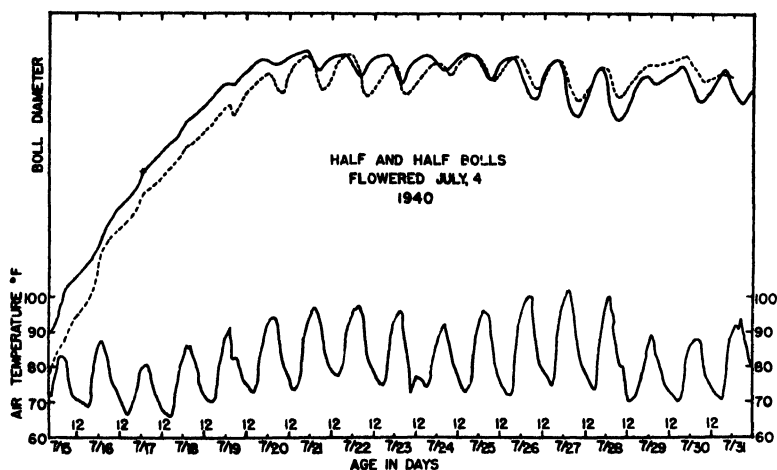
FIG. 7. Variations in the diameters of two cotton bolls starting 11 days after flowering and ending 16 days later. When young, the bolls do not shrink during the warm part of the day but as soon as they attain full size a marked shrinkage occurs in the middle of the day. Rain prevented shrinkage on July 29.

of the boll during its enlargement phase and those that occur after the cells have attained their mature dimensions is strikingly apparent. During enlargement the higher temperatures of midday increased the rate of growth but as soon as the cells reached their maximum size the higher temperatures of midday resulted in conspicuous shrinkage.

Severe drought decreases the daily increments of growth without changing its general pattern. Young bolls continue to enlarge more rapidly during the day, even though the plants are severely wilted, than at night when moisture conditions are more favorable. The total amount of each day's increase in size is less, however, than that which occurs in bolls that undergo their enlargement when the water supply is more adequate. The duration of the period of enlargement is also less in very dry weather (15 days) than when water is not a limiting factor (16 to 18 days). The effect of dry

weather in decreasing both the daily increments óf growth and the period during which increase in size occurs, is to reduce the size of the mature bolls. Mature bolls which underwent the enlargement phase of their growth during the exceptionally dry weather of late July, 1942, were only about half the size of bolls formed in the same plants earlier and later in the season when the plants were not subject to severe wilting.

Boll enlargement and fiber elongation are parallel processes that terminate at about the same time. Shortening the period of boll enlargement should, therefore, shorten the cotton fibers. STURKIE (11) has shown previously that fibers developed during periods of water stress are distinctly shorter than fibers formed when the period of enlargement has not been curtailed by deficient water. Insofar as the effect of soil water upon fruit size is concerned, cotton seems similar in its response to lemons (2), apples (10), peaches (6), pears (4) and many other fruits.

Discussion

Shrinkage in the size of vegetative and reproductive organs is a very common result of deficient water. Often as in the case of the full sized cotton bolls discussed in this paper the shrinkage is merely a temporary consequence of rapid transpiration, low water absorption or both. The diurnal variations in the diameter of lemon fruits reported by BARTHOLO-MEW (2) and those found by MacDOUGAL (8, 9) to occur in various fruits and even in good sized tree trunks seem essentially similar to the variations here reported for full sized cotton bolls. The shrinkage observed in full sized cotton bolls at the time of wilting of the parent plant is undoubtedly the result of a movement of water out of the boll to the leaves and other organs where the diffusion pressure deficits of the cells exceed those of the boll. Such an internal redistribution of water is a very common phenomenon in plants and is thoroughly familiar to all plant physiologists.

The continued enlargement of the young bolls during periods in which the parent plant exhibited severe wilting was not anticipated and seems contrary to the general experience of plant physiologists. It has long been recognized that the exposure of plants to full sunlight inhibited elongation and the fact has commonly been ascribed to a reduction in the turgor of the cells in the region of enlargement [LOOMIS, (7)]. In cotton, however, water apparently continues to enter the young bolls when it is being lost by full sized bolls, leaves and other vegetative organs. MacDOUGAL (8) has reported that young growing cells of sunflower stems continued to increase in size when cells in mature regions were shrinking as a result of deficient water. This fact, as MacDOUGAL points out, makes it necessary to consider carefully the cellular organization of any tissue before attempting to evaluate the effect of deficient water upon tissue enlargement. The shrinkage of a stem at midday does not necessarily indicate a stoppage of cell enlargement. Young cells in the stem may continue their enlargement but the increase in size may be completely masked by the decrease in size of the mature cells. The number of mature cells so greatly exceeds the

number of enlarging cells that the net results of deficient water will be a decrease in size even though enlargement of young cells continues uninterruptedly. In cotton bolls between the date of flowering and about 16 days later, the cells of the carpel wall and of the immature seeds continue to enlarge and hence to receive water at the expense of more mature cells. As soon as the bolls reach an age of 16 days the cells have reached their mature dimensions and no longer seem able to maintain their turgor when the plant is subjected to stress conditions.

The explanation of the high water absorbing capacity of the enlarging bolls cannot be considered in detail in this report but it does not seem to be the result of any simple osmotic mechanism. The cells of immature cotton seeds and carpel wall do not appear to have higher diffusion pressure deficits than the more mature cells of bolls that have completed their enlargement. The osmotic pressures and diffusion pressure deficits of enlarging and full sized cotton bolls will be discussed in a subsequent paper.

The importance that the high water absorbing capacity of enlarging bolls may have in affecting the water relations of the plant were strikingly revealed during the extremely dry weather of late July and early August 1942. Cotton plants of the same variety, but differing in age by about two weeks, were growing in adjacent rows in the field. The older plants had relatively few enlarging bolls while most of the bolls on the younger plants were in the enlargement phase of growth. The leaves of the younger plants wilted earlier than those of the older plants and by midday the greater severity of the wilting of the younger plants was conspicuous. In the older plants the bolls served as reservoirs from which water moved into the wilting leaves but in the younger plants the enlarging bolls continued to extract water from the vegetative tissues thus accentuating the low turgor produced by the high transpiration rate.

Summary

1. Continuous records of the diurnal and seasonal variations in the diameters of cotton bolls of different varieties have been recorded during the summers of 1940, 1941, and 1942.

2. The enlargement of young bolls is not inhibited by severe wilting of the parent plant.

3. Full sized bolls shrink in size when the parent plants are visibly wilted and regain their size during the night if low soil moisture is not a limiting factor.

4. The degree of shrinkage of full sized bolls is, in general, proportional to the severity of wilting of the parent plant.

5. During periods of severe drought shrinkage occurs later in the day and recovery during the night is only partial or even entirely absent.

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LITERATURE CITED[1]

1. ANDERSON, D. B., and KERR, T. Growth and structure of cotton fiber. Ind. & Eng. Chem. **30**: 48–54. 1938.

2. BARTHOLOMEW, E. T. Internal decline of lemons III. Water deficit in lemon fruits caused by excessive leaf evaporation. Amer. Jour. Bot. **13**: 102–117. 1926.

3. FURR, J. R., and TAYLOR, C. A. Growth of lemon fruits in relation to moisture content of the soil. U. S. Dept. Agr. Tech. Bull. 640. 1939.

4. HENDRICKSON, A. H., and VEIHMEYER, F. J. Irrigation experiments with pears and apples. California Agr. Exp. Sta. Bull. 667. 1942.

5. HUNT, G. M. Effectiveness of moisture—excluding coatings on wood. U. S. Dept. Agr. Circular 128. 1930.

6. JONES, I. D. Preliminary report on relation of soil moisture and leaf area to fruit development of the Georgia Belle peach. Proc. Amer. Soc. Hort. Sci. **28**: 6–14. 1931.

7. LOOMIS, W. E. Daily growth of maize. Amer. Jour. Bot. 21: 1–6. 1934.

8. MacDOUGAL, D. T. Hydration and growth. Carnegie Inst. Washington. Pub. no. 297. 1920.

9. ——————. Studies in tree growth by the dendrographic method. Carnegie Inst. Washington. Pub. no. 462. 1936.

10. MAGNESS, J. R., DEGMAN, E. S., and FURR, J. R. Soil moisture and irrigation investigations in eastern apple orchards. U. S. Dept. Agr. Tech. Bull. 491. 1935.

11. STURKIE, D. G. A study of lint and seed development in cotton as influenced by environmental factors. Jour. Amer. Soc. Agron. **26**: 1–24. 1934.

POLLEN GERMINATION AND POLLEN TUBE GROWTH, AS INFLUENCED BY PURE GROWTH SUBSTANCES[1]

Fredrick T. Addicott

Investigators studying the germination of pollen and growth of pollen tubes have, in the past, of necessity concerned themselves with restricted aspects of the problem. Most of the reports now in the literature deal with pollen germination as it has affected some aspect of cytology, genetics, or plant breeding. As a result, our knowledge of the physiology of pollen tube growth is far from complete; the need for a thorough understanding of the subject, however, is readily recognized. Recently a few reports of work with certain growth promoting substances on pollen germination have appeared (1, 3, 6). This paper extends previous work, and presents the results of experiments on germination of pollen and the growth of pollen tubes in response to 33 pure growth substances and several impure preparations. To the investigator's knowledge this work is the first in which any of the substances have been tested in the presence of optimum concentrations of boron, now known to be essential to the germination of the pollen of many species.

The germination of pollen and growth of the pollen tube are complex phenomena involving several morphological and physiological processes. Morphologically there is in germination a swelling of the pollen grain followed by protrusion of the tube. The tube nucleus usually remains in the cytoplasm near the tip of the tube. The generative cell may enter the tube from a few minutes to many hours after the appearance of the tube, depending on the species. Its division into two sperm nuclei usually takes place after entrance into the tube. *In vivo* the tube grows from the stigma to the vicinity of a female gametophyte and bursts, liberating its three nuclei. The distance it grows may be from a few millimeters to nearly one hundred. *In vitro* germination may approximate that on the stigma, but it is doubtful if normal tube growth has yet been obtained. Tubes often show various abnormalities. They may be short, granular, vesicular, thin, bifurcated, or various combinations of these.

Physiologically, the pollen tube requires several types of nutrients: (a) it requires water; (b) it requires inorganic salts; (c) it requires a source of energy (usually supplied as sugar); (d) it requires a complement of growth factors of hormonal or vitamin nature. Pollen may, however, supply many of these requirements from its own reserves. There is apparently considerable variation between species in this regard. Some pollen can germinate in distilled water (1). Other types, such as the pollen of Milla and Tropaeolum used in this investigation, apparently require nutrients from all four of the above categories.

Materials and methods

The pollen of two species was selected for the experiments: *Milla biflora*, a monocotyledon; and *Tropaeolum majus*, variety Golden Gleam, a dicotyledon. These were chosen because they represented the two principal groups of flowering plants, and their flowers could be obtained throughout the period available for experimentation.

A number of methods were tested for growing pollen tubes *in vitro*. Those involving agar or gelatin did not permit as ready observation during the period of growth as did the hanging drop method. The method involving hanging drops on the under side of the cover of a Petri dish was finally selected. Eight drops of the medium to be tested were placed on the cover and about 1 ml. of distilled water in the bottom of the dish, to maintain a saturated atmosphere. The drops had an average volume of

TABLE I

EFFECT OF THE COMPONENTS OF THE BASIC MEDIUM ON GERMINATION AND GROWTH OF THE POLLEN TUBE IN MILLA

MEDIUM	PERCENTAGE GERMINATION*	POLLEN TUBE LENGTH*
	%	%
Distilled H_2O	3	42
Distilled $H_2O + 0.01\%$ H_3BO_3	0	0
Distilled $H_2O + 12\%$ glucose	3	42
HOAGLAND and ARNON'S sol'n (less boron)	0	0
" " " " $+ 0.01\%$ H_3BO_3	8	42
" " " " $+ 12\%$ glucose	45	47
" " " " $+ 0.01\%$ $H_3BO_3 + 12\%$ glucose	100	100

* These values are expressed in terms of the controls taken as 100 per cent. In this series the actual value of the controls were: germination 62 per cent., tube length 2.3 diameters of the pollen grain. Each value is based on 60 to 120 pollen grains.

0.005 ml. which was obtained through the use of a fine pipette. Pollen from Milla was placed in four of the drops and pollen from Tropaeolum in the other four.

From 15 to 30 pollen grains were placed in each drop. In the case of Milla, pollen was taken from flowers which had opened within the past 12 hours. In the case of Tropaeolum, whose anthers open much later than the petals, pollen was taken from anthers which had opened in the past 12 hours. It was found that the viability of the pollen decreased with age. The size of the drop and the amount of inoculum were kept as constant as possible. It was noted that pollen tube growth was greatly increased when the number of grains was high in proportion to the amount of medium present. Since this phenomenon was presumably due to a diffusible chemical, the inoculum was kept low so that the chemical would be detected if it were among those added to the medium.

The basic medium contained water double distilled from Pyrex glass, HOAGLAND and ARNON'S solution I (supplying all the known inorganic

nutrients of plants) in which the boric acid content had been raised to 0.01 per cent. (5), and 12 per cent. glucose. The latter two concentrations were found to be optimum for both Milla and Tropaeolum. To this basic medium were added various pure growth, factors and impure preparations in at least five different concentrations. The boric acid requirement is in accordance with the findings of SMUCKER (7), COOPER (3), and WEBB (unpublished data). Five or more concentrations of glucose, sucrose, fructose, and glycerol were tested as sources of carbohydrate. Optimum germination and tube growth were obtained with 12 per cent. glucose in both species.

In recording results the percentage of germination and the length of the tubes were taken two hours after inoculation. Growth always ceased

TABLE II

EFFECTS OF VARIOUS IMPURE PREPARATIONS ON GERMINATION AND GROWTH OF THE POLLEN TUBE IN TROPAEOLUM AND MILLA

PREPARATION ADDED TO BASIC MEDIUM	CONCENTRATION	PERCENTAGE GERMINATION*		POLLEN TUBE LENGTH*	
		TROPAE-OLUM	MILLA	TROPAE-OLUM	MILLA
		%	%	%	%
Pollen	To cover the surface of the drops	133	141	156	181
Intact stigma	For Milla ⅓ stigma per drop was used	91	102	270	112
Minced stigma		91	92	65	79
Minced style		95	34	43	74
Stigma exudate of Milla	About 10%	131	104	186	163
Stigma exudate of Milla	100%		120		850
Beef extract (Difco)	1 mg. per liter	97	95	73	89
Peptone (Difco)	1 mg. per liter	96	94	167	99
Spanish saffron	80 mg. extracted with a liter of basic medium	149	140	183	115

* These values are expressed in terms of the corresponding controls which were taken as 100 per cent. in each case. Each value is based on 60 to 120 pollen grains.

before this time had elapsed. As no constant temperature apparatus was available the cultures were kept at room temperature. This was always close to 25° C. and never at any time during the experimental periods did the temperature vary more than 2° from 25° C. The length of the tubes was measured in terms of pollen grain diameters. This was a convenient unit which was quite uniform. The pollen grains of Milla have more than twice the diameter of those of Tropaeolum. The figures in the tables are based on averages from each experiment employing from 50 to over 100 pollen grains. The data in the tables have been expressed in terms of percentages of the amount of germination or growth in the "control" of each experiment. The "control," or basic medium, consisted of the salt solution and sugar mentioned above.

Observations

The effects of various factors in the basic medium are shown in table I. The value of glucose and a high concentration of boric acid is apparent.

Table II shows the effects of impure preparations and extracts when added to the basic medium. The observations of VASIL'EV (8), GOTOH (4) and others that the stigma and its exudate are active in promoting the growth of the pollen tube are demonstrated. That this growth, however, is not due entirely to the boron content of the stigma is apparent from the

TABLE III

EFFECTS OF PURE GROWTH FACTORS ON GERMINATION AND TUBE GROWTH OF TROPAEOLUM POLLEN

GROWTH SUBSTANCE CONCENTRATION
MILLIGRAMS PER LITER

GROWTH SUBSTANCE	100		10		1.0		0.1		0.01	
	GERMI-NATION	TUBE LENGTH	GERMI-NATION	TUBE LENGTH	GERMI-NATION	TUBE LENGTH	GERMI-NATION	TUBE LENGTH	GERMI-NATION	TUBE LENGTH
	%	%	%	%	%	%	%	%	%	%
Thiamin	95	66	90	244*	90	104	112	111	93	85
Niacin	53	45	80	90	94	104	77	110	81	110
Niacinamide	80	114	96	164	79	126	89	95	93	74
Riboflavin	98	145	94	100	90	76	98	91	95	112
Para aminobenzoic acid	48	33	94	96	84	84	94	84	97	100
Inositol	95	79	99	84	93	91	95	70	108	164
Pyridoxin	97	80	99	68	94	77	54	74	77	77
Ascorbic acid	103	41	92	115	94	81	96	69	92	124
Alpha-naphthyl acetamide	38	99	85	89	53	109	106	213*	109	64
Indoleacetic acid	4	33	92	91	91	114	78	62	96	137
Traumatic acid	14	31	54	31	101	77	109	65	70	49
Uric acid	114	85	117	139	88	126	56	139	97	107
Adenine	90	116	103	125	100	107	98	86	103	83
Thymine	64	83	66	45	107	49	79	114	67	91
Cytosine	117	168	117	101	93	123	130	76	78	127
Uracil	103	132	56	94	84	89	88	92	103	89
2 methyl-4,6 dihydroxy-purine	112	67	95	54	83	81	85	51	87	81
2 methyl-4-hydroxy-5-hydroxymethylpurine	68	159	71	113	100	68	86	83	74	117
2 methyl 4 amino 5-thio-formamidomethylpurine	30	117	34	136	92	53	95	99	119	76
2 methyl 4-hydroxy-5-aminomethylpurine-hydrochloride	0	0	74	79	112	87	64	121	79	114
2 methyl-4 amino-5-amino-methylpurine-hydro-chloride	93	125	77	79	67	110	73	136	86	106
Pimelic acid	3	49	74	49	129	59	91	64	96	79
Allantoin	78	92	35	58	90	86	89	92	89	83
Alloxan	79	95	74	80	102	95	80	125	90	92
2-chloroisothiamin-iodide	93	92	100	75	121	97	55	97	113	78

* Significant deviations from the controls.

fact that the medium already contained the optimum concentration of boric acid. Since it also contained the full complement of inorganic chemicals known to be essential to plant growth it seems highly probable that the effects of the stigma are due at least in part to one or more of its organic constituents. This suggestion is supported by the fact that an extract of saffron also shows some activity.

Germination and pollen tube growth were also promoted by crowding the pollen. This appears to be parallel to the well known "bios effect" in yeast and other microorganisms where the growth of organisms is stimulated when the inoculum contains large numbers. This effect in pollen has

TABLE IV

EFFECTS OF PURF GROWTH FACTORS ON GERMINATION AND TUBE GROWTH OF MILLA POLLEN

GROWTH SUBSTANCE	GROWTH SUBSTANCE CONCENTRATION MILLIGRAMS PER LITER									
	100		10		1.0		0.1		0.01	
	GERMI-NATION	TUBE LENGTH	GERMI-NATION	TUBE LENGTH	GERMI-NATION	TUBE LENGTH	GERMI-NATION	TUBE LENGTH	GERMI-NATION	TUBE LENGTH
	%	%	%	%	%	%	%	%	%	%
Thiamin	75	51	87	59	77	67	72	57	94	53
Niacin	96	90	92	71	53	127	85	80	86	68
Niacinamide	86	56	97	49	110	34	88	74	64	102
Riboflavin	118	142	87	112	95	107	107	208*	112	142
Para-aminobenzoic acid	164*	80	78	214*	203*	117	193*	104	163*	110
Inositol	176*	153	190*	123	161*	110	180*	153	149	141
Pyridoxin	109	72	106	105	88	83	43	139	78	244*
Ascorbic acid	92	98	105	84	100	80	102	110	94	87
Alpha-naphthyl acet-amide	23	110	62	146	105	110	115	139	103	160
Indoleacetic acid	67	61	82	142	74	178	74	244*	82	107
Traumatic acid	0	0	13	55	62	117	100	133	84	117
Uric acid	67	31	90	41	97	63	51	20	67	57
Adenine	106	94	108	76	109	50	110	68	112	60
Thymine	102	128	102	94	93	168	94	188*	102	87
Cytosine	76	51	89	98	96	104	100	76	97	109
Uracil	137	107	142	139	153*	79	108	79	137	170
2-methyl-4,6-dihy-droxypurine	140	99	127	123	142	103	130	95	149	91
2-methyl-4-hydroxy-5-hydroxymethyl-purine	98	168	97	114	98	141	98	101	98	128
2-methyl-4-amino-5-thioformamido-methylpurine	102	154	98	228*	95	114	96	248*	99	168
2-methyl-4-hydroxy-5-aminomethylpurine-hydrochloride	99	262*	100	141	99	74	95	188*	98	101
2-methyl-4-amino-5-aminomethylpurine-hydrochloride	99	202*	99	141	102	322*	99	154	99	101
Pimelic acid	106	110	112	110	114	102	108	110	108	110
Allantoin	110	57	99	102	105	86	105	78	112	65
Alloxan	103	39	104	44	108	52	112	60	104	70
2-chloroisothiamin-iodide	0	0	11	71	19	191*	124	147	148	167
Ca-pantothenate	110	130	84	99	73	57	124	205*	126	213*

* Significant deviations from the controls.

also been recently noted by BECK and JOLY (1). With both species no tropistic growth was observed either towards or away from clumps of pollen. The direction of growth appeared to be completely random, regardless of the arrangement of the pollen.

TABLE V

EFFECTS OF PURE GROWTH FACTORS ON GERMINATION AND TUBE GROWTH
OF TROPAEOLUM POLLEN

GROWTH SUBSTANCE	GROWTH SUBSTANCE CONCENTRATION (IN TERMS OF SATURATION)									
	SAT'D		0.1 SAT'D		0.01 SAT'D		0.001 SAT'D		0.0001 SAT'D	
	GERMI-NATION	TUBE LENGTH	GERMI-NATION	TUBE LENGTH	GERMI-NATION	TUBE LENGTH	GERMI-NATION	TUBE LENGTH	GERMI-NATION	TUBE LENGTH
	%	%	%	%	%	%	%	%	%	%
Alpha-tocopherol	98	56	113	96	85	81	91	107	108	107
Estrone	129	130	110	92	129	115	95	121	126	143
Xanthine	98	141	133	94	64	89	46	98	92	120
Guanine	129	83	100	76	116	101	115	55	128	82
4 methyl-5-hydroxymethyl uracil	124	152	119	74	77	117	42	130	100	74

Tables III to VI present the results of tests of pure substances. The starred (*) values represent significant deviations from the controls. Significant differences were found to be an increase in germination of 51 per cent. and an increase in tube length of 85 per cent. over the controls. The terms "significant difference" as here used indicates that analysis showed that there was less than 1 possibility in 20 that the observed difference was due to pure chance. Sixteen of the substances tested affected germination or tube growth when judged on this basis.

The above results were all from tests with single substances. Table VII shows the results of experiments with two mixtures of pure substances.

TABLE VI

EFFECTS OF PURE GROWTH FACTORS ON GERMINATION AND TUBE GROWTH
OF MILLA POLLEN

GROWTH SUBSTANCE	GROWTH SUBSTANCE CONCENTRATION (IN TERMS OF SATURATION)									
	SAT'D		0.1 SAT'D		0.01 SAT'D		0.001 SAT'D		0.0001 SAT'D	
	GERMI-NATION	TUBE LENGTH	GERMI-NATION	TUBE LENGTH	GERMI-NATION	TUBE LENGTH	GERMI-NATION	TUBE LENGTH	GERMI-NATION	TUBE LENGTH
	%	%	%	%	%	%	%	%	%	%
2-methyl-1,4-naphtho-quinone	8	46	5	46	92	109	142	114	129	180
Alpha-tocopherol	98	66	93	103	106	79	106	74	91	79
Estrone	84	60	85	120	108	74	95	86	98	55
Xanthine	65	37	93	101	112	90	108	103	107	94
Guanine	82	27	105	140	127	203*	141	257*	129	138
4-methyl-5-hydroxy-methyluracil	86	81	97	89	102	140	90	144	102	127
Acenaphthene	126	154	147	197*	139	199*	63	90	153*	130

' Significant deviations from the controls.

These combinations did not appreciably add to germination or tube growth over that of single substances.

Table VIII presents a classification of all the pure substances tested and indicates which were effective in promoting germination or pollen tube growth.

TABLE VII

EFFECTS OF MIXTURES OF PURE SUBSTANCES ON GERMINATION AND GROWTH OF POLLEN TUBES

CONCEN-TRATION		SUBSTANCE	PERCENTAGE GERMINATION		TUBE LENGTH	
			TROPAE-OLUM	MILLA	TROPAE-OLUM	MILLA
1	mg./l.	Indoleacetic acid				
10	mg./l.	Niacinamide				
10	mg./l.	Thiamin				
100	mg./l.	Riboflavin	150	112	80	147
0.01	mg./l.	Inositol				
0.1	sat'd	Estrone				
0.01	sat'd	Guanine				
0.1	mg./l.	Indoleacetic acid				
0.1	mg./l.	Niacin				
0.1	mg./l.	Thiamin				
0.1	mg./l.	Riboflavin				
0.1	mg./l.	Inositol				
0.1	mg./l.	Pyridoxin		43		147
0.1	mg./l.	Ascorbic acid				
0.1	mg./l.	Para-aminobenzoic acid				
0.1	mg./l.	Ca-pantothenate				
2%		Gum Tragacanth				
To give pH 4.4		Citric acid				

Discussion

From these experiments it appears that germination of the pollen grain and the subsequent growth of the pollen tube are not necessarily related phenomena. They can be stimulated independently. For example, inositol increased the germination of Milla pollen up to 90 per cent: over that of the controls without greatly affecting the length of the tubes. Guanine, on the other hand, increased the length of the pollen tubes of Milla up to 157 per cent. more than that of the controls, without significantly affecting the percentage of germination. Two substances, para-amino-benzoic acid, and acenaphthene affected both processes.

It is of interest to note that several classes of substances show activity in the germination and growth of pollen. These include vitamins, plant hormones, and pyrimidines and purines. The latter group of substances should perhaps be classified with the plant hormones since the purines are active as leaf growth factors. They were kept separate, however, for convenience in handling a large group of chemically distinct compounds. Several vitamins are known to be growth hormones in the higher plants or growth factors for the lower plants. The ultimate function of these chem-

TABLE VIII

SUMMARY OF THE PURE SUBSTANCES EMPLOYED IN EXPERIMENTS ON THE GERMINATION
AND GROWTH OF POLLEN TUBES, AND THEIR ACTIVITY

SUBSTANCE	SIGNIFICANT INCREASES			
	PERCENTAGE GERMINATION		TUBE LENGTH	
	TROPAEOLUM	MILLA	TROPAEOLUM	MILLA
Water soluble vitamins				
Thiamin†			**	
Niacin†				
Niacinamide†				
Riboflavin				*
p Aminobenzoic acid		**		*
Inositol		**		
Pyridoxin†				**
Ascorbic acid				
Ca pantothenate				*
α-Naphthyl acetamide	.		*	
Oil soluble vitamins				
2-methyl-1,4-naphthoquinone				
α-Tocopherol				
Plant hormones				
Indoleacetic acid				**
Traumatic acid				
Animal hormones				
Estrone				
Pyrimidines and purines				
Uric acid				
Adenine				
Xanthine				
Guanine				**
Thymine				*
Cytosine				
Uracil		.		
2 methyl-4,6-dihydroxypurine				
2 methyl-4-hydroxy-5-hydroxymethylpurine				
2 methyl-4-amino-5-thioformamidomethyl-purine			.	**
4 methyl-5-hydroxymethyluracil	.	.		
2 methyl-4-hydroxy-5-aminomethylpurine-hydrochloride	.			**
2 methyl-4-amino-5 aminomethylpurine-hydrochloride	.			**
Miscellaneous compounds				
Pimelic acid				
Allantoin				
Alloxan				
2-chloroisothiamin-iodide				*
Acenaphthene				*

* Significant activity, less than 1 chance in 20 that the results were due to pure chance.

** Highly significant activity, less than 1 chance in 100 that the results were due to pure chance.

† These may also be classified as plant hormones.

icals within the pollen has not yet been determined, but it is reasonable to suppose that it is similar to the function of the same chemical in other parts of the plant. That is, indole-acetic acid is essential to cell elongation, and the various water soluble vitamins function in respiratory enzyme systems. The purines have been reported to promote leaf growth under some circumstances (2).

Even with the addition of the growth substances employed, the growth of the pollen tube can neither be considered normal nor approaching that of pollen germinated in stigma exudate. In exudate the tubes averaged 16 diameters long. In the various media employed tubes varied from 1 to 6 or 7 diameters long. Tubes in synthetic media were often thin, burst soon after germination, or were sometimes highly vesicular. This would indicate that the media employed were incomplete and lacking in some other factor(s) since it can be supplied at least in part by stigma exudate. A search is now under way for this factor(s). The results will be reported in a later communication.

Summary

This paper reports the results of tests of 33 pure growth substances on the germination of pollen and the growth of pollen tubes of Tropaeolum and Milla. Sixteen substances increased germination or tube growth significantly.

The results give evidence supporting the view that the germination of pollen and the growth of the pollen tube are at least in part physiologically independent. Certain substances may affect one process and not the other.

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LITERATURE CITED

1. BECK, WILLIAM A., and JOLY, RUSSELL A. Some growth phenomena in cultured pollen tubes. Trans. Amer. Mic. Soc. 60: 149–162. 1941.
2. BONNER, DAVID M., and HAAGEN-SMIT, A. J. Leaf growth factors. II. The activity of pure substances in leaf growth. Proc. Nat. Acad. Sci. 25: 184–188. 1939.

3. COOPER, WILLIAM C.   Vitamins and the germination of pollen grains and fungus spores.   Bot. Gaz. **100**: 844–852.   1939.

4. GOTOH, KAZUO.   Physiological researches on pollen with special reference to the artificial germination of Gramineae pollen. ₒMem. Fac. Sci. Agr. Taihoku Imp. Univ. **3**: 61–197.   1931.

5. HOAGLAND, D. R., and ARNON, D. I.   The water-culture method for growing plants without soil.   Univ. California Agr. Exp. Sta. Circular 347.   1939.

6. SMITH, PAUL F.   Studies on the growth of pollen with respect to temperature, auxins, colchicine, and vitamin B_1.   Amer. Jour. Bot. **29**: 56–66.   1942.

7. SMUCKER, THEODOR.   Über den Einfluss von Borsäure auf Pflanzen inbesondere keimende Pollenkörner.   Planta **23**: 264–283.   1934.

8. VASIL'EV, IU. P.   Studies on the effect of stigmatic secretions and of the ovule on pollen germination of certain plants.   Bot. Jour. U.S.S.R. **19**: 321–337.   1934.

AN AUTO-IRRIGATOR FOR GROWING PLANTS IN THE LABORATORY

A. D. MOINAT

(WITH ONE FIGURE)

At present, the most practical method of controlling soil moisture for the roots of laboratory plants seems to be the method of adding water to the surface soil in order to bring the soil container, its soil, and the plants growing therein to a certain predetermined weight which represents the desired moisture content. The principal difficulty in this procedure lies in attaining the original distribution of moisture in the soil which was quite uniform at first, but soon afterward becomes variable both vertically and horizontally through the soil container. HENDRICKSON and VEIHMEYER (2) found that uniform distribution of moisture in a soil container is unattainable after drying, until the water content again reaches the rather high level marked by the moisture equivalent. At low moisture contents, there are regions in the container with variable water content and the most active roots will be in the region of optimum moisture.

The most successful mechanism for moisture control seems to be that devised by LIVINGSTON (3) and further developed in a new device, the double walled pot, by RICHARDS (4). Here, the plants in the irrigator must pull against the weight of the column of supply water through a tube reaching to a reservoir at a lower level than the irrigator itself. In the double walled pot, RICHARDS and LOOMIS (5) have found a decreasing degree of control of the soil moisture the lower one departs from the moisture equivalent. At the lower moisture contents, the water is lost from the container much more rapidly than it can be replaced because of the low rate of water movement in dry soil. The soil in the container may reach the wilting percentage and the plants growing therein remain unwilted if their roots are in contact with the source of supply through the porous clay walls of the container.

In the apparatus described in the present paper, a much simpler system of water control was used, although one which has its limitations. At low moisture percentages there is difficulty in supplying moisture at a rate sufficiently high to maintain the certain desired low soil moisture which represents the actual water available to the roots of plants growing in the irrigator.

Materials and methods

The principle involved in all irrigators which attempt to control the water supply for roots, involves the separation of the roots from actual contact with the water supply. Several separating substances have been tried, such as the unglazed clay used by LIVINGSTON (3) and others, and the plaster-of-Paris and unglazed clay plates used in this study.

In the first trials, porous clay plates of rather uniform 3/16-inch thick-

ness, sealed with plaster-of-Paris in the bottom of tin soil containers (tops of gallon pails) were used. Nine of these were placed on the surface of sand of a rather fine textural grade, which had been uniformly compacted into a large 30×30-inch rectangular, galvanized iron container, 18 inches deep. The water came up from a supply in the bottom of this container, through the sand, to the porous plate in the bottom of each soil container. The water was maintained at an approximately constant level by the use of feed tubes from a flask above, using the Mariotte principle. Distribution across the bottom of the galvanized iron sand container was secured by using a layer of coarse gravel about $1\frac{1}{2}$ inches deep over the bottom, upon which the sand was packed. Three levels of sand can be used in one sand container, but due to the need for more uniform light for all of the plants in one test, it is probably better to use sand at a single level in each container and that level to be near the top of the container.

Heavy 28-inch dish pans were also used as sand containers with different rates of supply secured by filling each of the several pans with one of several different textural grades of sand.

A second type of plate developed consisted of plaster-of-Paris mixed thin and poured over a compacted smooth sand in such a sand container as a glazed earthenware jar of 2- or 3-gallon capacity. Soil was then placed on the plate to fill the upper part of the jar. Great care in any case must be observed to get complete sealing of the plate with the walls of the soil container (fig. 1). The plaster-of-Paris plate formed in this fashion is not as uniform in thickness as the manufactured clay plate but has the advantage of giving excellent contact with the sand, which seemed to be a drawback of the manufactured plate.

The soil moisture has been determined by two methods: first, sampling and oven drying to constant weight at 105° C.; and, second, by use of the Bouyoucos (1) electrical resistance method. With the latter method, the plaster-of-Paris blocks in which the resistance electrodes were imbedded were laid either directly on the plaster-of-Paris plate of the irrigator or, in the majority of cases, on a thin layer of soil above the plate. The resistance was then measured with a Leeds and Northrup conductivity apparatus.

Results

POROUS CLAY PLATE IRRIGATORS

The results of a series of irrigator studies using the large galvanized iron sand containers and the porous clay plates as described above, are given in table I. The sand in the irrigator was approximately 30 cm. deep. Irrigator 4 in the table was in reality a new set-up of number 3 in which the soil was replaced in the soil containers, but the containers themselves were not moved. Gradual drying of the soil containers is seen in the 3A–3C, and 4A–4B columns although this drying is not entirely uniform. It is evident from the table that there is considerable variation between the various containers of one irrigator, a divergence which is probably too great except in

studies where rather wide ranges of soil moisture are permissible. The moisture in the sand beneath the plates is also variable and often does not correlate exactly with the moisture in the soil containers above. This variation in sand moisture, which is undoubtedly not entirely due to errors in sampling, will always be a cause of some variability in moisture supply to the irrigator plates.

STONE JAR IRRIGATORS AND PLASTER-OF-PARIS PLATES

Eight stone jars were arranged as irrigators as illustrated in figure 1. Irrigator number III was set up with provision for daily reading of the electrical resistance and conditions for obtaining a gradual decline in the

TABLE I

SOIL MOISTURE IN POROUS CLAY PLATE IRRIGATORS SET UP IN 30 × 30-INCH SAND CONTAINERS NOS. 2–4

Soil CONTAINER	IRRIGATOR TANKS						
	2A	3A	3B	3C	4A	4B	Sand*
	%	%	%	%	%	%	%
1		21.5	21.0	18.3	16.9	12.0	3.46
2	13.1	19.6	17.8	16.2	16.4	12.1	3.24
3	13.4	18.9	17.9	14.7	17.6	13.3	3.56
4	11.4	18.8	18.5	17.3	18.8	15.8	3.84
5	12.8	18.0	16.8	17.4	19.4	13.2	3.97
6	13.3	18.1	18.9	15.9	17.0	12.4	3.79
7	12.4	20.17	18.7	17.9	18.3	13.0	3.77
8	14.3	20.4	20.5	18.3	19.2	15.6	4.9
9	11.2	23.5	22.3	17.9	16.3	12.1	3.34
Mean	12.7	20.2	19.2	18.7	18.1	13.3	3.77
Em†	0.40	0.58	1.76	0.31	0.39	0.49	0.17
Date started	3–16	4–7			5–19		
Date sampled	4–1	5–7	5–14	5–19	5–25	6–9	6–15

* Sample taken under plates of 4B.
† Em: standard error of the mean.

moisture content of the soil. There was no disturbance of the soil for sampling until the apparatus was dismantled at the end of eleven days. Two resistance blocks were placed in the soil, one on either side of the irrigator. From table II, it can be seen that the resistance gradually increased, but somewhat irregularly. The final moisture determined from a soil sample taken from the upper surface of the two resistance blocks was 22.3 and 22.4 per cent., respectively. Although several of the irrigators in this series were set up with the view to obtaining readings at a low moisture content, none reached a low point in the time available because of the amount of water needed to pour the plaster-of-Paris plate.

Irrigator number IV was set up to run with a high moisture supply. The resistance increased in the 10-days' run rather irregularly and the moisture samples from on top of the blocks were approximately equal as is shown in table II. The resistance readings in all of the present stone-jar-irrigator

series are somewhat variable because of the high moisture in the soil. In dry soil, the end points for the hummer on the resistance apparatus are more definite.

Irrigators V, VI, and VII were rather irregular in their resistance readings, which did not always seem to follow exactly the decreases in soil moisture as determined by sampling. This irregularity may have been due to disturbance of the soil when the samples were taken, or to the fact that the resistance readings are more variable at the higher moistures.

In general, resistance readings appear to be less accurate at the higher soil moistures, and each resistance block must be calibrated in order to make
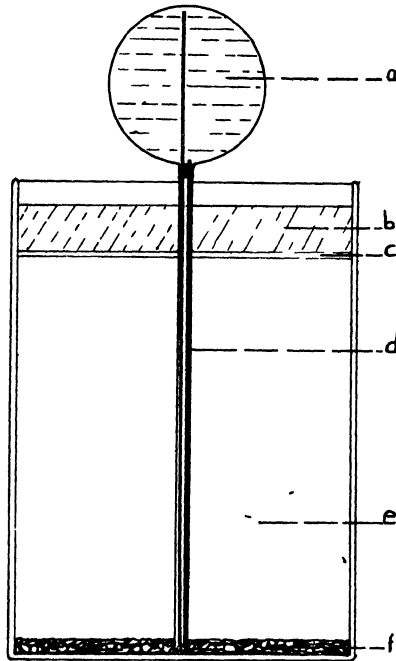
Fig. 1. Auto irrigator with plaster of-Paris plate: a, reservoir; b, soil; c, plaster-of Paris plate; d, conduit for water supply tubes; e, capillary column of sand; f, gravel for distribution of water across bottom of container.

comparisons between blocks. It is believed, however, that this method has definite possibilities in certain types of work since the soil need not be disturbed while following moisture changes.

DISTRIBUTION OF MOISTURE IN THE STONE JAR IRRIGATORS USING PLASTER-OF-PARIS PLATES

From samples taken at different positions in the irrigator soil some idea of the uniformity of water supply can be gained. In general, there is slightly more water in the soil at the surface of the plaster-of-Paris plate than there is on top of the resistance block about a half inch higher in the irrigator. See table III, irrigator V.

TABLE II

ELECTRICAL RESISTANCE IN OHMS AND PERCENTAGE OF SOIL MOISTURE IN STONE JAR IRRIGATORS

DATE*	III				IV				V		VI				VII			
	Block 5		Block 4		Block 3		Block 4		Block 8		Block 6		Block 7		Block 9		Block 10	
	ohms	%	ohms	%	ohms	%	ohms	%	ohms	%	ohms	%	ohms	%	ohms	%	ohms	%
7	309		510															
7	494		521															
8	529		583		587		583											
8	561		604		609		604											
9	566		611		602		611											
10	589		604		619		605		560		347	26.7	260	25.0	514		599	
11	621		688		634		677		531		398	24.7	305	24.7	553		614	
12	559		591		611		615		500	25.8	374	22.7	276	23.5	486	29.4	490	28.2
13	579		515		633		620		477		409		276		517	27.4	549	
14	583		617		653		631		520		377		288		588		657	
16	606		627		700		666		487		387	23.1	300	23.86	587	26.6	710	
17	595	22.3	627	22.4	666	32.2	672	32.3	496	24.6	369	22.7	293		619	25.8	677	25.4
18	575		631		465		457		475		363		297		619		451	25.8

* All readings were taken in August on the dates indicated.

TABLE III

VARIATION IN MOISTURE CONTENT OF SOIL AT DIFFERENT POSITIONS IN THE IRRIGATORS V–VIII

IRRIGATORS

V			VI			VII			VIII		
DATE*	POSITION†	H₂O	DATE*	POSITION	H₂O	DATE*	POSITION	H₂O	DATE*	POSITION	H₂O
		%			%			%			%
12	block 8	25.8	11	block 6	26.7	12	block 9	29.4	13	1	28.3
12	1, plate	26.3	12	block 6	24.7	13	block 9	27.4	13	2	27.7
12	block 8	25.4	12	3	24.2	14	2	27.3	13	3	27.4
13	3	26.4	13	block 6	22.7	16	block 9	26.6	14	2a	28.9
13	2	25.8	14	2	23.2	17	block 9	25.8	14	1a	27.3
13	2a plate	26.2	16	block 6	23.1	12	block 10	28.2	16	2	28.7
14	1a	22.8	17	block 6	22.7	13	block 10	28.2	16	3a	29.7
14	2	25.9	11	1a tube‡	21.5	14	block 10	26.9			—
16	3a plate	25.1	11	3a	26.2	16	block 10	25.4			—
16	block 8	24.9	12	2a	25.0	17	block 10	25.8			—
17	block 8	24.6	12	block 5	24.7	17	2	28.5			—
17	2a plate	25.6	12	block 5	23.5						
			13	3	25.7						
			14	1	20.0						
			16	block 5	23.9						
			17	....	23.0						

* All samples were taken in August on the dates indicated.

† Position, refers to the point in the irrigator where the soil was sampled. Six loci for samples were numbered in the sequence 1, 1a, 2, 2a, 3, and 3a around the irrigator about 1 inch from the outer wall. One resistance block was placed in irrigator V at position 1; two were located in irrigators VI and VII at positions 1 and 2a. Block, in the table indicates that the sample was taken from the upper surface of the block which had been given a certain number. Plate, indicates that samples were taken on the upper surface of the plaster-of-Paris plate. All other samples, except where otherwise indicated, were taken at the same level as the upper surface of the block.

‡ A full depth sample taken with a tube. All other samples taken with a spatula from a layer about ¼-inch thick.

The average difference in moisture percentages between comparable positions in irrigator VI is 0.7 per cent., while the maximum difference is 1.7 per cent. in soil of an irrigator that was rather frequently disturbed by sampling. In irrigator VII the average difference in water content of the soil from different positions was 1.05 per cent. with a maximum difference of 1.4 per cent. The average difference in irrigator VIII is 1.15 per cent. with 2.4 per cent. as the maximum.   The largest differences are in the third and last sampling after some disturbance of the soil.

With the porous clay plate irrigators the average difference of comparable samples as taken from the sand containers, or tanks 2, 3 and 4 reported above, was 0.74 per cent. but was found to be over 2 per cent. in individual soil containers.

NUTRIENT SOLUTION IN THE IRRIGATOR

In stone jar irrigators numbers I and II, washed Ottawa sand was used above the plate instead of soil and the same type of sand was used for the capillary column below the plate.   In irrigator I, nutrient solution was used instead of water; in irrigator II, distilled water was used for the water supply.   Beans were planted in both irrigators.   The plants which were grown in the irrigator supplied with distilled water for 3 weeks began to show deficiency symptoms in the form of reduced growth and lighter colored areas on the leaves.   The plants which had been supplied with the same quantity of a complete nutrient solution appeared to be healthy and made good growth in spite of the fact that the nutrients had to move through the $CaSO_4$ plate.   One or two small roots managed to get through the plate of each irrigator but they were too small to account for the difference in growth. Further studies will be necessary to determine the value of this method of nutrient supply.

Discussion

In the irrigator which has been described we have a simple device by means of which one can grow plants at various rates of water supply.   At the lower moisture levels, the roots obtain their moisture directly from the porous plate rather than from the soil but, nevertheless, it is possible to get the desired effects on the plants.   The soil over the roots, of course, does not contain as much moisture as is actually available to roots which are in contact with the plate and often massed on the plate.   Covering the soil surface to prevent excessive evaporation would tend to delay the exhaustion of moisture from the irrigator soil but would also introduce aeration problems. The writer and others have used this method to obtain plants with the characteristics typically developed under different moisture supplies.   In some cases florists' pots, with the drainage hole sealed with plaster-of-Paris, and then sunk in water supplying sand, have been used instead of porous plates.

Summary

1. An irrigator is described which depends upon the capillary rise of

water through sand to supply the water for plants growing in soil above a porous plate embedded in the surface of the sand.

2. By varying the depth of the sand or its textural grade, moderate sized plants can be grown under conditions of high, low, and moderate moisture supply.

3. Specifications to obtain a definite soil moisture are, for all practical purposes, impossible to list at present because of the variability in the sand, the porous separation plate, the soil, etc.

4. In a set-up of stone jar irrigators, with the soil moisture ranging between 20 and 30 per cent., the distribution of moisture across the irrigator was uniform within 2.5 per cent.

5. At the lower soil moistures, approaching the wilting point, soil samples from soil even very close to the plate may not represent the moisture available to plants with their roots in direct contact with the plate.

6. The Bouyoucos electrical resistance apparatus offers a method of following soil moisture conditions in the irrigator without disturbing the soil. The accuracy of this method proved to be less at the higher moisture contents.

The idea of the capillary column of sand and the porous separating plate for the irrigator described in this report was received from DR. C. F. HOTTES of the University of Illinois. The author also wishes to express his appreciation for suggestions and aid given by DR. S. V. EATON and MR. RORERT WHITMORE in the course of the resistance measurements which were carried out at the Barnes Laboratory of Plant Physiology, University of Chicago.

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LITERATURE CITED

1. BOUYOUCOS, G. J., and MICK, A. H. An electrical resistance method for the continuous measurement of soil moisture under field conditions. Michigan State College. Agr. Exp. Sta. Tech. Bull. 172. 1940.

2. HENDRICKSON, A. H., and VEIHMEYER, F. J. Moisture distribution in soil in containers. Plant Physiol. 16: 821–826. 1941.

3. LIVINGSTON, B. E. Porous clay cones for the auto-irrigation of potted plants. Plant World 21: 202–208. 1918.

4. RICHARDS, L. A. Usefulness of capillary potential to soil moisture and plant investigators. Jour. Agr. Res. 37: 719–741. 1928.

5. ——————, and LOOMIS, W. E. Limitation of auto-irrigators for controlling soil moisture under growing plants. Plant Physiol. 17: 223–235. 1942.

A METHOD OF INCREASING THE RATE OF SEED GERMINATION OF *TARAXACUM KOK-SAGHYZ*[1, 2]

J. LEVITT AND P. C. HAMM

(WITH THIRTEEN FIGURES)

The germination of kok-saghyz seed is a gradual process, the first seedlings pushing through in a few days, the rest appearing successively during a period of several weeks. Obviously, surface soil moisture must be maintained high throughout this period if a good stand is to be established, especially since the seeds are small and have to be sown very shallow. Since such ideal conditions seldom prevail, and since the long drawn out germination period is highly conducive to damping off, poor stands are frequently obtained. Some means of speeding up the process of germination is therefore desirable.

In view of the rapid appearance of the first few seedlings, and in the absence of hard seed coats, a plausible explanation of the tardiness of the remaining seeds is that they are not fully mature, *i.e.*, their physiological and perhaps morphological development was not completed when they were harvested.

The aim of this investigation was to find some way to mature these seeds before sowing them. This should be possible by increasing the moisture content of the seeds sufficiently to permit the necessary physiological changes, but insufficiently to permit germination. It should then be possible to dry the seeds so that they can be sown in the usual way.

The two recommended methods (presoaking and vernalization) wittingly or unwittingly make use of this principle. The former, however, permits some germination, and in both cases the seeds are sown wet. Consequently injury sometimes results. Furthermore, it is difficult to obtain uniformity in water uptake by these methods. Some better procedure must therefore be sought.

In the following investigation, unless otherwise stated, each value for germination is an average of three Petri plates containing 100 seeds per plate. The seeds were sown on a piece of filter paper which covered the bottom of the plate, and were supplied with 5 ml. of water. Since temperature was not controlled (except when stated), individual experiments cannot be compared directly with each other.

The amount of water absorbed by the seeds can be accurately controlled by permitting the seeds to come to equilibrium with a solution of known concentration. Seeds were therefore soaked in dextrose solutions of different concentrations, rinsed with water, dried for about 8 hours, and sown. The stimulation was distinct and none of the seeds were injured by the treatment

(fig. 1). Not all of the concentrations tried were of equal value. There was an optimum range, (M dextrose), on either side of which the effect decreased.

If the effect of the solution is simply osmotic, it should be possible to
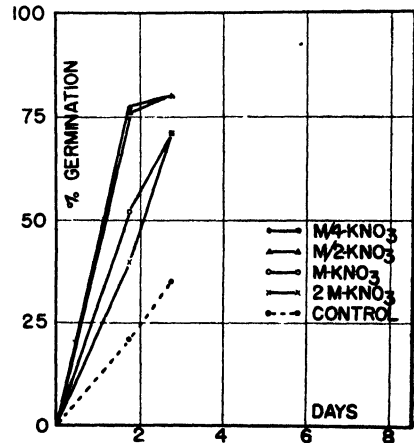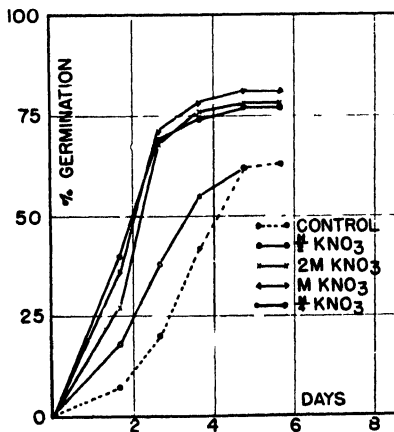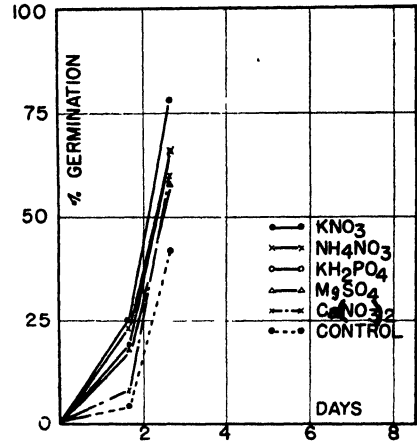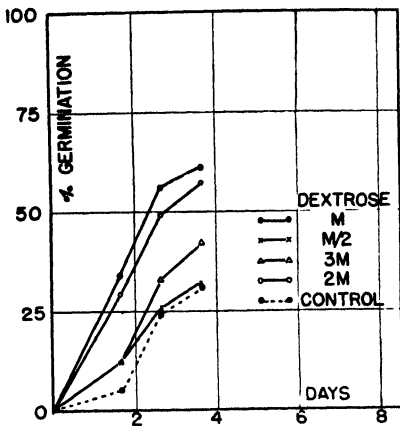
FIGS. 1–4. Upper left: Seeds soaked for 3 days in dextrose solutions, rinsed, dried for 8 hours, then sowed.

Upper right: Seeds soaked for 3 days in salt solutions, rinsed, dried for 8 hours, then sowed.

Lower left: Seeds soaked for 3 days in KNO_3 solutions, rinsed, dried for 8 hours, then sowed.

Lower right: Seeds soaked for 3 days in solutions, rinsed, then sowed without previous drying.

duplicate these results with other solutes. This would be desirable since even the stronger dextrose solutions become mouldy. Mineral nutrients were next tried, in the hope that they might also have a stimulating effect on the seedlings due to their nutrient value.

All the solutions tried increased the rate of germination, some more than others (fig. 2). The differences are not necessarily due to the chemical nature of the solutes, since though the molar concentrations were the same, the osmotic values were not.

Decreasing the concentration increased the stimulation up to a point (fig. 3), just as in the case of dextrose. Apparently, if the water content
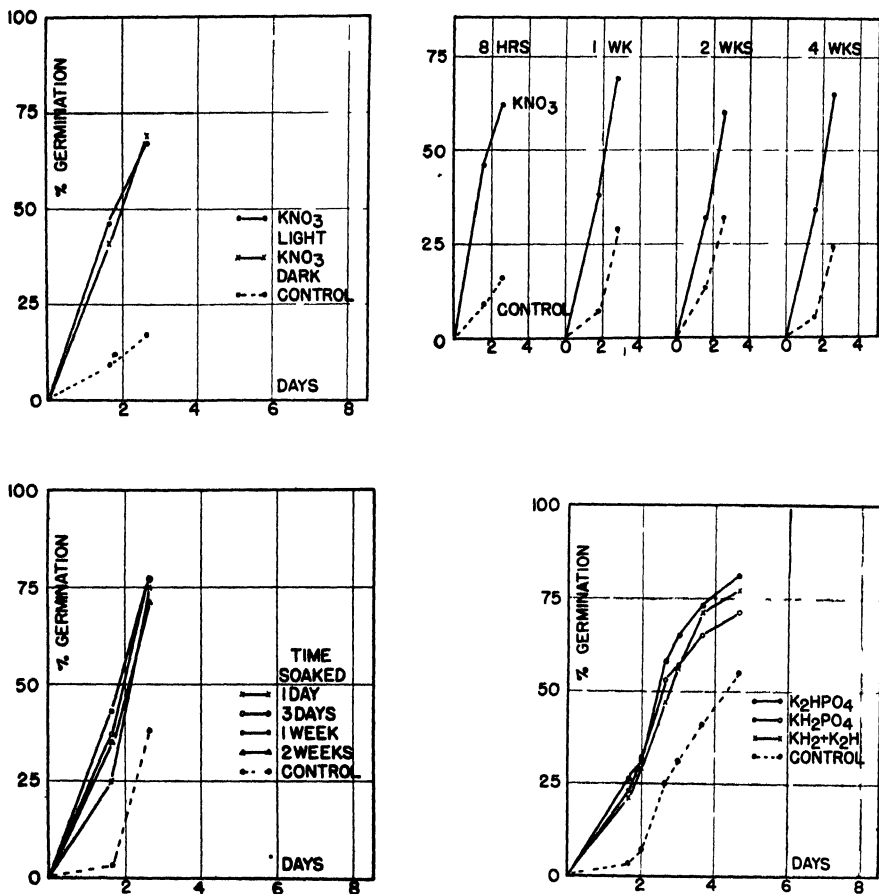
FIGS. 5–8. Upper left: Seeds soaked for three days in M KNO$_3$ one set in the light, the other in the dark. Both rinsed, dried, then germinated in the dark.

Upper right: Seeds soaked for 3 days in M KNO$_3$, rinsed, dried for 8 hours, 1 week, 2 weeks, and 4 weeks, respectively, then sowed.

Lower left: Seeds soaked in M KNO$_3$ for 1 day, 2 days, 1 week, and 2 weeks, respectively, then rinsed, dried 8 hours, and sowed.

Lower right: Seeds soaked in M KH$_2$PO$_4$, M K$_2$HPO$_4$, and a 1:1 mixture of the two, respectively, for 3 days, then rinsed, dried, and sowed. pH's of solutions, 4.1, 8.9, and 7.6.

of the seeds is increased too much, the effect is lost during the subsequent drying process, even though no sign of germination is evident. When the same experiment was repeated without drying the seeds, the lowest concentration produced the greatest stimulation (fig. 4).

The effect of other factors on the stimulation produced by the osmotic method was next investigated. Treatments were found to have the same effect whether conducted in the light or in the dark (fig. 5).

From the practical point of view, it is of prime importance to know whether the effect is retained for long periods after drying. Figure 6 shows that it is.

Surprisingly short soaking periods proved to be as effective as considerably longer ones (fig. 7). The longest periods seemed somewhat less effective.
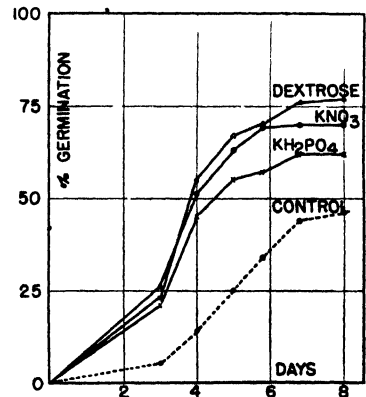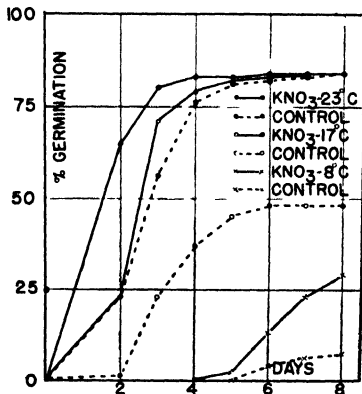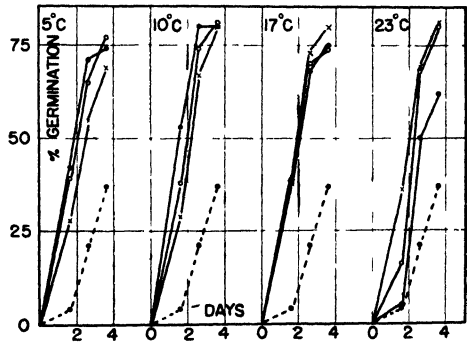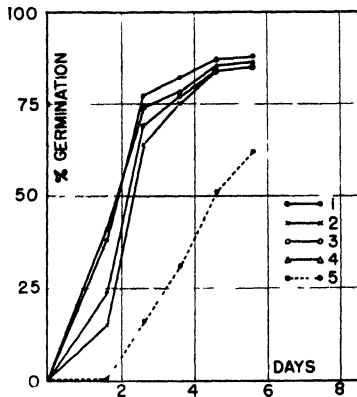
FIGS. 9–12. Upper left: Seeds soaked 3 days in M KNO$_3$, then rinsed once, (1); sowed without rinsing (2); washed for two hours (3); and rinsed three times with 10 ml. water (4). All were then dried and sowed. Untreated control (5).

Upper right: Seeds soaked in M KNO$_3$ at 5° C., 10° C., 17° C., 23° C., then rinsed, dried and sowed. ×———×, soaked 4 days; O———O, soaked 1 week; ●———●, soaked 2 weeks.

Lower left: Seeds soaked 3 days in M KNO$_3$, rinsed, dried, then sowed at 23° C., 17° C., 8° C., respectively. Similar results were obtained at 4° C., germination beginning after 2 weeks.

Lower right: 100 seeds soaked 3 days in M dextrose, M KNO$_3$, M KH$_2$PO$_4$ respectively, then rinsed, dried, and sowed in soil (about ⅛–¼ inch deep).

It was thought that since pH affects the hydrophily of colloids, this factor might have some influence on the degree of stimulation. Monobasic and dibasic potassium phosphate were therefore compared with each other and with mixtures of the two. The differences were slight (fig. 8).

The amount of washing after soaking had very little effect when KNO_3 was the solute used (fig. 9). More toxic solutes, however, (e.g. $MgSO_4$, NH_4NO_3) must be removed more thoroughly, or the effect is lost and even a retardation of germination, or death of the seeds, may result.

For practical as well as theoretical reasons, it is important to know the relationship of the stimulation to temperature. Figure 10 shows that the length of the soaking period required to produce the effect varies inversely with the temperature, as would be expected. The temperature coefficient, however, is low enough (below 2) to indicate that a physical factor is limit-
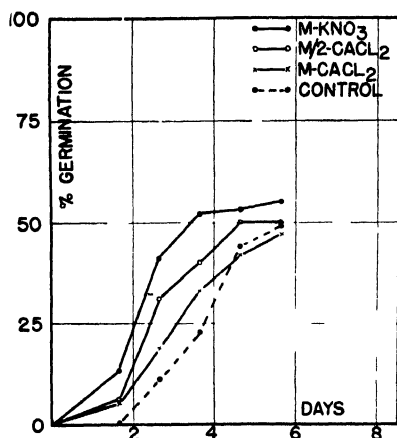
FIG. 13. Seeds exposed to atmospheres in equilibrium with M and M/2 $CaCl_2$ for 1 week, then sowed at the same time as control set and set soaked 3 days in M KNO_3, rinsed and dried.

ing. It is interesting to note that at 23° C. the 4-day treatment is best, at 5 and 10° the 2-week period is best, at 17° C. the three lengths of treatment had the same effect.

The effect persists regardless of the temperature during germination, at least from 4 to 23° C. (fig. 11). One surprising difference is, however, revealed. At 23° C. only the rate of germination is increased by the treatment, at 17° C. both the rate and the total percentage of germination, at least at the end of 3 weeks.

All these tests were performed in Petri plates. It is, of course, essential to know whether the same effect is obtained in soil. Figure 12 shows that it is.

Some other kinds of seeds were tested to determine whether the stimulating effect of the osmotic method is specific to kok-saghyz.

Kentucky bluegrass seed was soaked for 8 days in M KNO_3, washed, then dried before sowing. Little or no germination occurred in either the treated

or the control seeds, as long as they were kept in 'the dark. On transfer to the light, however, the treated seed germinated to the extent of 98 seeds in 300, the control 7 seeds in 300, in a period of one month. Rutabagas, on the other hand were apparently injured by the treatment and germination was markedly reduced.

If the osmotic method owes its effectiveness simply to an osmotic control of the amount of water taken up by the seeds, similar results should be obtainable by simply exposing the seeds to an atmosphere of the same vapor pressure as the solution. Seeds were therefore exposed to the atmosphere over solutions of $CaCl_2$ for a week, then sown (fig. 13). This method induced a stimulation, though not as great as that obtained by the osmotic method. The difference may perhaps be due to slowness of absorption of water vapor.

Summary

Germination of kok-saghyz seed was hastened by permitting the seeds to absorb water in quantity insufficient to induce germination, then drying the seed before sowing. The stimulation was obtained whether the uptake of water was controlled osmotically or by exposure to atmospheres of definite relative humidity.

The following results were obtained with the osmotic method. No loss of the stimulation occurred even 4 weeks after drying. The optimum concentration was M/2 for KNO_3, M for dextrose. When the seeds were sown without being dried, even weaker concentrations were optimum. Two weeks' treatment proved better than shorter periods at 5 and 10° C., 4 days was better than 1 or 2 weeks at 23° C., whereas at 17° C. all three lengths of time gave the same results. Two weeks at 10° C. gave the best results of all the above. The Van't Hoff coefficient was less than 2. When germinated at 23° C., the treatment had no effect on the final percentage of germination. At lower temperatures it increased the final percentage of germination. Factors having little or no effect on the stimulation are: the nature of the solute (if thoroughly washed off), the amount of washing (in the case of KNO_3), the pH of the solution, the presence or absence of light.

The stimulation was obtained whether the seeds were germinated in Petri plates or in crocks of soil.

INHIBITING INFLUENCE OF THE LEAVES ON THE PHOTOPERIODIC RESPONSE OF NOBEL SPINACH

ALICE P. WITHROW, R. B. WITHROW, AND
J. P. BIEBEL

(WITH TWO FIGURES)

Introduction

CAJLACHJAN (2), MOSKOV (9), LUBIMENKO (8), HAMNER and BONNER (3), HAMNER and LONG (4) and BORTHWICK and PARKER (1) have shown that the flower forming stimulus in short day plants is initiated in the leaves. One leaf of a normal plant kept in a favorable short photoperiod was reported to be sufficient to induce flowering in the short day plants, Biloxi soybean (1) and Xanthium (3), even though the remaining leaves on the plant were kept in an unfavorable photoperiod. Under certain circumstances, however, involving two-branched plants or grafted plants where one branch or plant is induced to flower while the other is kept in an unfavorable photoperiod, the leaves on the latter branch or plant have been shown to inhibit flowering of that branch or plant. The effect is not carried over to other plant portions (1, 3, 5).

Work of this type on long day plants has not been very extensive. KNOTT (6) reported that the flower stalk formation in Virginia Savoy and Old Dominion varieties of spinach was not affected by the photoperiod given the bud but only by that received by the leaves. LANG and MELCHERS (7) reported that leafy biennial *Hyoscyamus niger* plants which were pre-treated with a low temperature of 5° C., flowered only in a long photoperiod and failed to flower in a short photoperiod or in darkness. If, however, all the leaves were removed from plants which had received a low temperature treatment, they flowered under long photoperiods, short photoperiods, or in darkness. This indicates that the failure of this species to flower in a short photoperiod or in darkness may be caused by an inhibiting effect exercised by the leaves.

Methods and results

PRELIMINARY EXPERIMENTATION

A preliminary experiment with Nobel spinach was run to determine the reactions of this variety to photoperiodic treatment of the bud and the leaves. The plants were grown in flats of a sand-peat mixture and were supplied with a complete nutrient solution. Vegetative plants which had been kept in a 10-hour photoperiod from germination were used for these and subsequent studies. A night temperature of 60° F. was maintained with a minimum day temperature of 70° F. Part of the plants were left intact and part were defoliated so that only the last matured leaf remained. The defoliation

technic was used when later experimentation showed that leaves in an un-
favorable photoperiod exercised an inhibiting influence on flower bud
initiation. It was thought desirable to reduce the number of such leaves to
a minimum.

The intact plants were given two treatments, 24 plants per treatment,
using essentially the same technic as that described by KNOTT (6). In the
first treatment, the plants were covered with opaque rubberized black cloth so
as to maintain a 10-hour photoperiod. The covering was perforated with
holes 16 mm. in diameter with iron washers centered over the holes to help
hold the cloth in place. The holes were so placed as to expose the bud and a
few adjacent young leaves. The plants were then placed under a 24-hour
photoperiod (continuous irradiation) made up of solar radiant energy
supplemented with 20 footcandles of incandescent lamp radiant energy.
This was used for all subsequent long photoperiod treatments. The buds
and a few adjacent young leaves, therefore, received 24-hour photoperiods
while the remainder of the plant received 10-hour photoperiods.

The second treatment was begun 38 days later, using vegetative plants
of the same age as in the first treatment. The plants were placed under
the long photoperiod with the entire plant exposed. Final data were taken
52 days after the first treatment was begun.

The one-leafed plants received three treatments, 16 plants per treat-
ment: (1), the entire plant was given a 24-hour photoperiod; (2), the bud
was given a 10-hour photoperiod and the leaf a 24-hour photoperiod; and (3),
the bud was given a 24-hour photoperiod and the leaf a 10-hour photo-
period. Individual squares of opaque rubberized black cloth were so
arranged that the indicated portions of the plants received the indicated
photoperiod. Dissection under a low power binocular microscope was made
at the end of the experimental period where macroscopic buds were not
visible.

No intact plants formed flower buds except those in which the leaves
received a long photoperiod, confirming KNOTT's results with Virginia
Savoy and Old Dominion varieties of spinach. Where the plants were
defoliated to one leaf and the bud was given a long photoperiod while the
leaf received a short photoperiod, the plants also failed to form flower buds.
If only the leaf received the long photoperiod and the bud was given a short
photoperiod, the results were the same as when both the leaf and bud were
given a long photoperiod, *i.e.*, all the plants flowered.

INHIBITING INFLUENCE OF THE LEAVES

In an endeavor to determine if the presence of leaves in an unfavorable
photoperiod inhibits flower bud initiation, the flowering of intact plants with
only a portion of the plant in a favorable photoperiod and of partially
defoliated plants was studied.

Intact plants with approximately nine leaves were given three treat-
ments. A number of leaves developed during the experimental period,

increasing the initial number.   The treatments were: (1), one leaf exposed
to a 24-hour photoperiod while the remainder of the plant was given a short
photoperiod by covering with opaque rubberized cloth at the end of 10 hours
of solar irradiation; (2), three leaves exposed to a long photoperiod with the
remainder in a short photoperiod; (3), the entire plant exposed to a long
photoperiod.   The method of curtaining the plants is shown in figure 1.

The plants were grown in subirrigation gravel culture with a complete
nutrient solution.   The minimum night temperature was approximately 60°
F. with a minimum day temperature of 70° F.   Twenty-four plants were
used for each treatment.   The long photoperiod was applied for 26 days
after which time the plants were harvested.   The plants were 58 days old
at the beginning of the experiment.   Microscopic dissection was made to
observe floral primordia at the close of the experiment.

FIG. 1.   Method used to curtain intact plants so that one and three leaves received a
24-hour photoperiod while the remainder of the plant received a 10-hour photoperiod.

No flower primordia could be found at the end of the treatment when
only one leaf received a long photoperiod.   When three leaves were given a
long photoperiod, no macroscopic buds developed; but on microscopic exami-
nation, 70 per cent. of the plants were found to have developing floral
primordia.   If the entire plant received a long photoperiod, all the plants
had macroscopic buds at 17 days; the first buds were visible in 12 days.

In the defoliation experiments, plants with about nine leaves were
defoliated respectively to one and three leaves and left intact.   The leaves
left on the plant were the youngest mature leaves and were more or less
symmetrically arranged about the bud when three remained.   The older
leaves and the young developing leaves were removed.   Young developing
leaves were removed daily from the partially defoliated plants after the
beginning of the experimental treatments.   The plants were placed in a
24-hour photoperiod immediately after defoliation.   The same number of
leaves received the long photoperiod as was the case with the intact plants.
The cultural conditions, age and number of plants, selection of leaves for the

long photoperiod treatment, length of treatment, and method of observation for flower buds were the same as for the intact plants.

The plants defoliated to one and three leaves all had macroscopic flower buds 19 days after the beginning of the treatment, about two days later than did the intact plants.   The plants with one leaf first showed floral buds 16 days after the beginning of the treatment and those with three leaves, 13 days.   The rate of flower stalk development was greater, in direct proportion to the number of leaves on the plant.   It should be pointed out that the leaf area eventually exposed to the long photoperiod was not in proportion to the number of leaves since those plants having only one or three leaves produced much larger leaves than did the intact plants.   Figure 2 shows plants in which one and three leaves of partially defoliated and intact plants received 26 long photoperiods.

Fig. 2.   Flower stalk formation resulted when Nobel spinach plants were defoliated to one and three leaves and given a long, 24 hour photoperiod treatment (left plants of each pair).   When corresponding numbers of leaves of intact plants were given long photoperiod treatments (right plants of each pair), those with one leaf exposed to the long photoperiod failed to form floral primordia.   Those with three leaves exposed formed floral primordia but no flower stalks during the experimental period.

Discussion

The results secured indicate that the bud of Nobel spinach is relatively insensitive to photoperiod and that the phasic development of the bud is controlled principally by reactions in the leaf.   Unlike Biloxi soybean (1) and Xanthium (3), one leaf of a normal intact plant exposed to a favorable photoperiod was not sufficient to cause flower bud initiation if the remainder of the plant was given a short photoperiod.   One leaf, however, was sufficient in the absence of leaves in an unfavorable photoperiod, indicating that leaves in an unfavorable photoperiod exert an inhibiting influence on flower bud initiation and development.   Even if three leaves, which approximated one-third of the leaves of the plant at the beginning of the treatment, were exposed to a long photoperiod treatment, the remainder of the leaves kept in an unfavorable photoperiod exerted an inhibiting influence on the expression of the flower forming stimulus initiated in those leaves which were in a long photoperiod.

Conclusions

1. The initiation of flower buds in Nobel spinach is controlled by the leaf and the bud is relatively insensitive to photoperiod.

2. Leaves kept in an unfavorable short photoperiod exert an inhibiting influence on flower bud initiation and development in Nobel spinach.

LITERATURE CITED

1. BORTHWICK, H. A., and PARKER, M. W. Photoperiodic perception in Biloxi soybeans. Bot. Gaz. 100: 374–387. 1938.
2. CAJLACHJAN, M. H. On the mechanism of photoperiodic reaction. Compt. Rend. (Doklady) Acad. Sci. U.R.S.S. 1: 89–93. 1936.
3. HAMNER, K. C., and BONNER, J. ·Photoperiodism in relation to hormones as factors in floral initiation and development. Bot. Gaz. 100: 388–431. 1938.
4. ————————, and LONG, E. Localization of photoperiodic perception in *Helianthus tuberosus*. Bot. Gaz. 101: 81–90. 1939.
5. HEINZE, P. H., PARKER, M. W., and BORTHWICK, H. A. Floral initiation in Biloxi soybean as influenced by grafting. Bot. Gaz. 103: 518–531. 1942.
6. KNOTT, J. E. Effect of a localized photoperiod on spinach. Proc. Amer. Soc. Hort. Sci. 31: (suppl.) 152–154. 1934.
7. LANG, A., and MELCHERS, G. Über den hemmenden Einfluss der Blätter in der photoperiodischen Reaktion der Pflanzen. Naturwiss. 29: 82–83. 1941.
8. LUBIMENKO, V., and BOUSLOVA, E. Contribution à la théorie du photoperiodisme. II. Compt. Rend. Acad. Sci. U.R.S.S. 14: 149–163. 1937.
9. MOSKOV, B. S. Rôle of leaves in photoperiodic reaction of plants. Bull. Appl. Bot. Gen. & Pl. Breed. Ser. A. 17: 25–30. 1936.

THE AMERICAN MISTLETOE WITH RESPECT TO CHLOROPHYLL AND PHOTOSYNTHESIS[1]

R. O. FREELAND

(WITH ONE FIGURE)

Introduction

In numerous botanical texts one may find a paragraph or so devoted to mistletoe. The context is very much the same whether the books are of old or recent vintage. The authors usually state, among other things, that mistletoe is parasitic upon the host plant for water and minerals but may be only partially parasitic with respect to food since it contains an abundance of chlorophyll and can make its own food.

It is obvious that American mistletoe, *Phoradendron flavescens,* is attached to the host and that it contains green plastids. HEINRICHER (2) found that the green pigment did not develop in mistletoe in the absence of light. But the author has never seen any published evidence that this group of plants can carry on photosynthesis or that the green pigment is chlorophyll. It is felt that the data included in this paper might help to substitute facts for speculation. It is also hoped that someone living in an area where mistletoe is common may follow up this work with ringing, dark-box, and other experiments which may clarify the problem of relative dependence or independence of mistletoe with respect to the host plant.

Green pigments

In the preparation of the leaves of mistletoe for extraction they were first dried at 80° C. and then powdered. The pigments were dissolved from 5 gm. of the leaf powder with warm 95 per cent. ethyl alcohol. The alcohol plus the pigments was then diluted to 82 per cent. with water. To this latter solution 200 ml. of petroleum ether were added. After being shaken the green pigments largely separated with the petroleum ether layer. Then to this ether fraction with the green pigments an equal volume of 92 per cent. methyl alcohol was added. After being shaken together these two solvents separated into two layers, each containing a fraction of the green pigments.

Each of the two solutions obtained was quite green in transmitted light and showed red fluorescence in reflected light. By trial it was found that one-half centimeter depths of each solution gave the most distinct differences in light absorption. So using this depth of solution and solvent, respectively, the extinction coefficients of each of these fractions were determined at short intervals in the visible portion of the spectrum. The results are recorded in table I and figure 1.

[1] Paper from the Department of Botany, Northwestern University.

TABLE I

EXTINCTION COEFFICIENTS (NEGATIVE LOGARITHM OF THE PERCENTAGE OF LIGHT TRANSMIS-
SION) FOR SOLUTIONS OF GREEN PIGMENTS OF AMERICAN MISTLETOE LEAVES.
(A) THE PETROLEUM ETHER FRACTION; (B) THE METHYL
ALCOHOL FRACTION

SPECTRUM	EXTINCTION COEFFICIENT		SPECTRUM	EXTINCTION COEFFICIENT	
	A	B		A	B
$m\mu$			$m\mu$		
440	1.28	2.42	590	0.20	0.27
450	1.24	1.90	600	0.32	0.43
460	1.20	1.95	610	0.29	0.40
470	1.12	1.95	620	0.31	0.40
475		1.61	625		0.50
480	0.69	1.12	630	0.39	0.58
485		0.65	635	0.67	
490	0.34	0.39	640	1.22	1.04
495		0.27	645		1.33
500	0.19	0.19	650	1.70	1.40
510	0.09	0.15	655	1.11	1.18
520	0.09	0.10	660	0.52	0.83
530	0.13	0.14	670	0.12	0.14
540	0.08	0.13	680	0.05	0.12
550	0.02	0.14	690	0.02	0.03
560	0.12	0.12	700	0.07	0.06
570	0.11	0.21	710	0.07	0.03
580	0.18	0.23	720	0.07	0.03
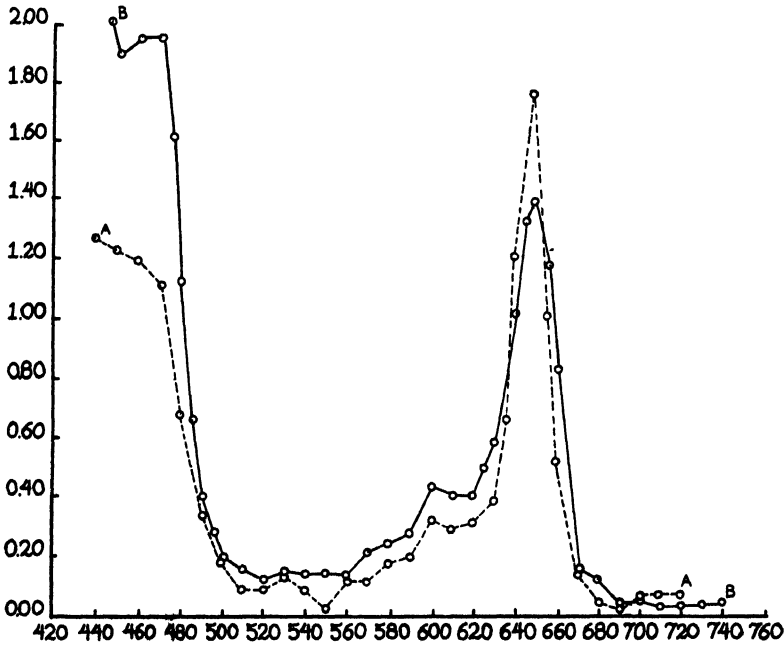
FIG. 1.   Curves for light absorption for solutions of the green pigments of American
mistletoe leaves.   A, petroleum ether fraction; B, methyl alcohol fraction.   Extinction
coefficient-values on the ordinate.   Wave length of light in $m\mu$ on the abscissa.   From
data in table I.

From what is known of the relative solubility of the chlorophylls, chlorophyll *a*, if present, should be in the petroleum ether fraction and chlorophyll *b* in the methyl alcohol. From the tabulated results it is clear that the green pigments in the methyl alcohol gave strong absorption bands at 440–445 and 645–655 mμ with lesser ones at 530 and 600–620 mμ; whereas the principal absorption bands for the pigments in the petroleum ether fraction were at 440–470 and 640–655 with lesser ones at 530, 560, and 600 mμ. The absorption bands for the two solutions are somewhat similar (fig. 1), except that the principal bands for the petroleum ether fraction are shifted toward the red end of the spectrum as compared with the methyl alcohol fraction.

A comparison of these results with the known absorption bands for chlorophyll leads the writer to conclude that chlorophyll *a* and chlorophyll *b* were present in the petroleum ether and methyl alcohol, respectively.

Photosynthesis

Determinations of carbon dioxide used or produced by mistletoe were made during the months of March, April, July, and August. Short sec-

TABLE II

DATA RELATIVE TO THE RATES OF PHOTOSYNTHESIS IN THE AMERICAN MISTLETOE, BASED UPON THE AMOUNT OF CO_2 USED. FROM 25 TO 50 CC. OF MISTLETOE WERE USED IN EACH EXPERIMENT

TRIAL	TEMPERATURE	TIME	CARBON DIOXIDE CONSUMPTION*	
			APPARENT	CALCULATED TOTAL
	C.°	hr.	mg.	mg.
1	29–33	2.5	0.0	1.8
2	32–37	3.0	1.5	4.5
3	29–33	2.5	5.0	6.8
4	33–38	3.0	4.0	7.0
5	25–37	3.0	0.0	2.0
6	36–35	3.5	0.0	3.3

* Milligrams CO_2 per 10 ml. mistletoe per hour.

tions of the host tree branches were sawed off, leaving the clumps of mistletoe intact. The amputated stems were submerged in water with the parasitic plants in the air. Under these conditions the plants were kept in the greenhouse and showed no signs of deterioration in the few days while they were being used. During the experiments the plants were inclosed in bell jars, through which a continuous flow of air passed, before going through absorption towers containing potassium hydroxide solution after the manner of HEINICKE and HOFFMAN (1). Thus changes in carbon dioxide were measured under the conditions of light and temperature incident in the greenhouse and then with the belljar covered with a light-proof hood. These latter measurements of respiration were considered advisable because of the rather large bulk of host stem in the experimental chambers. Although

the rates of respiration were probably not the same in the light and dark, the amount of apparent photosynthesis was corrected for these rates of respiration in an attempt to find the approximate total photosynthesis. The rates were all calculated on the basis of the total volume of leaves plus stems of mistletoe, since both are green. Obviously the values would be larger if only the leaves had been used as a basis for calculation.

Many determinations were made under different light intensities and temperatures such as normally occur from day to day in a greenhouse. The results of a few of these separate trials are given in table II. It will be noted that the values for apparent photosynthesis vary from zero to positive. Some of the low values for apparent photosynthesis were undoubtedly due to the large amount of respiration found for the section of host stem and mistletoe plants. The approximate total photosynthesis (apparent photosynthesis corrected for respiration) was always positive; therefore, the data indicate that this species of mistletoe can and does carry on photosynthesis.

Summary

1. The American mistletoe, *Phoradendron flavescens,* was examined with respect to the identification of the green pigments and possible photosynthesis.

2. Extinction coefficients for the green pigments indicate the presence of chlorophylls *a* and *b*.

3. Photosynthesis was found to occur in this species of mistletoe.

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LITERATURE CITED

1. HEINICKE, A. J., and HOFFMAN, M. B.   An apparatus for determining the absorption of carbon dioxide by leaves under natural conditions. Science n.s. **77** : 55–58. 1932.
2. HEINRICHER, E.   Chlorophyll-free growth of mistletoe caused by simultaneous lack of light and nutrients.   Biol. Abs. **5**: 13592.   1931.

BRIEF PAPERS

CHLOROSIS IN SUDAN GRASS[1]

F. L. WYND

Introduction

Sudan grass is one of the most important forage crops in the Rio Grande Valley, Texas. Growth is luxuriant and rapid, and the high yields suggest that the nutrients in the soil are eminently satisfactory for the growth of this species. Repeated cuttings may be made, often at intervals as short as 8 to 10 days, until 3 to 10 crops have been harvested. The leaves from the latter crops, however, become progressively more chlorotic until they become almost devoid of chlorophyll. Sometimes chlorosis appears in the second cutting, in which case the plants rarely survive more than 3 harvests. Other plantings do not become chlorotic until the third or even the fourth harvest, and in these instances the plants survive for a longer period. This chlorosis is one of the most serious diseases of Sudan grass in this area, and it often becomes a limiting factor in its production. For the past 3 years, the author has carried out field experiments in the vicinity of Elsa, Hidalgo County, Texas, in an effort to discover the cause of this nutritional disturbance. These experiments were designed particularly to test the validity of several *current theories concerning the causes of this disease. The study is incomplete, but the imminence of the writer's induction into military service and the importance of the problem itself has induced him to publish a brief statement of the present status of the study in the hope that it will aid students of the general problem of nutritionally induced chlorosis.

Experimentation

EFFECT OF SOIL REACTION

All of the soils of this area belong to the pedocal group. The pH values of water extracts vary from 7.2 to 8.2, depending on the amount and nature of the bicarbonate and carbonate salts present. This suggests that the alkalinity of the soil may cause chlorosis by rendering iron, and other trace elements, unavailable. The fact that the chlorosis of citrus trees in the area frequently disappears if the soil is treated with acidifying agents lends credence to this theory. Accordingly, field plots were treated with various amounts of ammonium sulphate, ammonium phosphate, and sulphur, and the results were observed over a period of 3 successive seasons. Although the pH of the upper layer of the soil was changed in degrees varying from 8.2 to 3.5, no effect on the chlorosis of Sudan grass was observed.

EFFECT OF FERROUS SULPHATE

A set of plots were arranged adjacent to those described above and vari-

ous amounts of ferrous sulphate were added in addition to the acidifying agents. Chemical analysis of the leaves showed that this caused an increased absorption of iron, but in no case was there any lessening of chlorosis.

EFFECT OF APPLYING TRACE ELEMENTS IN SPRAYS

Dilute solutions of iron, manganese, copper, zinc, and boron were applied singly and in combination by a hand spray. Applications were made twice to each crop, but this did not prevent chlorosis.

EFFECT OF THE CALCAREOUS LAYER IN THE PROFILE

It seems significant that the first crop produced is never chlorotic and it shows no sign of nutritional disturbance. This suggests that the roots are feeding in the upper, less calcareous layers during the early period of growth, but as the root system expands with age, they enter the lower, highly calcareous zone. The fact that the roots tend to follow the lowering zone adequately supplied with water tends to accentuate this downward penetration of those roots most active in absorption. Highly calcareous clay occurs at a depth of 24 to 36 inches in the soils which were included in the present study. This layer is rich in nutrient ions, but its high pH would seriously affect the availability of iron and other trace elements, hence the aerial growth produced during this period of growth might suffer from a lack of these substances. This theory was tested by carefully dissecting the soil profile at the time the first harvest was made, and before chlorosis had appeared. Even at this early age, when the plants showed no sign of nutritional disturbance, the roots had penetrated as deeply as 45 inches which was well into the layer of carbonate accumulation.

EFFECT OF CALCAREOUS CLAY

The experiment described above is not conclusive, since significant absorption undoubtedly occurred in the upper layers of the soil profile which were sometimes only slightly calcareous. A supply of highly calcareous clay from the lower levels of the profile was removed and mixed in various proportions with the top soil. In no case, did chlorosis appear earlier than in the plants growing in the undisturbed natural soil.

EFFECT OF REPEATED HARVESTS IN OTHER AREAS

It might be argued that the roots of Sudan grass are intrinsically unable to maintain a normal supply of nutrients to successive growths of tops. The roots and tops may become out of "balance" in some physiological relationship under the abnormal condition imposed by frequent removal of the tops. This theory was tested by growing Sudan grass near Midland, Douglas County, Kansas, on soils of varying base exchange capacity, and which contained various amounts of carbonates. Although some of these soils contained approximately the same amount of total replaceable bases as did the soils of Hidalgo County, Texas, and also sufficient carbonates to give

a soil reaction of pH = 8.0, the growth from successive cuttings did not become chlorotic.

EFFECT OF THE SOIL ITSELF

The results of the experiments described above suggest that there is some nutritional phenomenon associated with the intrinsic nature of the soils of the Lower Rio Grande Valley that induces chlorosis of Sudan grass. This possibility was tested by transporting topsoil from 10 of the most important soil series of Hidalgo County to Urbana, Illinois, where pot studies were conducted in the greenhouse. Six successive cuttings were taken during the summer and early autumn of 1942, and in no instance did chlorosis appear!

Discussion

The experiments described in this paper indicate, superficially, that the climate, and not the nutritional aspects of the soils of southern Texas produces chlorosis in the later cuttings of Sudan grass. Although the various aspects of climate undoubtedly affect the physiology of absorption, it seems more reasonable to suppose that climate is secondary to the effect of some, as yet unrecognized, aspect of the nutritional environment presented by the soil in producing the chlorosis described. There is no evidence at the present time concerning these causative factors.

Conclusions

Although Sudan grass grows luxuriantly in southern Texas, successive cuttings become progressively chlorotic. The plants finally become so devoid of chlorophyll that further growth is impossible. This condition is not due to the pH of the soil, to the availability of trace elements, to calcareous layers in the soil, nor to any intrinsic inability of the root to support continued vegetative growth. Climatic factors may prevent the appearance of chlorosis, although it is probable that climatic effects are secondary to some unknown nutritional property of the soil profile.

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ENZYME ACTION DOMINATED BY ASSOCIATED COLLOIDS

H. C. EYSTER

Recently EYSTER (1) showed that the adsorptive capacity of finely ground activated charcoal for methylene blue was reduced by ether, chloroform, sodium barbital, sulfanilamide, and saponin as well as by ethyl alcohol. This research was promoted by JOHNSON, BROWN and MARSLAND (3) who reported that luminous bacteria were partly or totally "blacked out" by the application of narcotics. In addition, EYSTER investigated the action of diastase on soluble starch and found that it, too, was reduced by the same narcotics. It was assumed that the narcotics decreased the adsorptive capacity of the enzyme for the substratum in a manner similar to the effect of narcotics on activated charcoal. The logical conclusion was that the results supported the argument that enzyme action is fundamentally adsorptive. This would then mean that the effect of narcotics on luminous bacteria was simply a reduction in the adsorptive capacity of the enzyme, luciferase.

Subsequently, GEIGER (2) cast doubt on the validity of those experiments for satisfactorily explaining the mechanism of narcosis of luminous bacteria. He based his opinion on the fact that "narcotics which retard the action of isolated diastatic ferment markedly increase the diastatic activity of liver cells." This instigated an investigation to bring about a correlation of these two apparently conflicting sets of observations.

The experiment described in table I was performed. In every case 50 ml. of 1 per cent. soluble starch, 5 ml. of a 1 per cent. diastase solution, and enough narcotic to give the stated concentration were diluted to 100 ml. with distilled water. The concentration for each narcotic was based on the final solution volume of 100 ml. The charcoal was kept dry and carbon-dioxide-free in a desiccator with $CaCl_2$ near the top and KOH on the bottom. The temperature of the digestive mixtures was kept at approximately 25° C.

As the table readily shows, ethyl alcohol, ether, and chloroform decreased the digestive action of diastase on soluble starch when the diastase was not associated with charcoal. When associated with charcoal, however, the digestive action was increased as reported for the liver cells. It appears that the charcoal adsorbs the enzyme and prevents it from acting freely on the soluble starch. It also appears that the narcotics, being more drastic in their effect on the adsorptive capacity of charcoal than on the adsorptive capacity of enzymes, release the enzyme from the charcoal particles and give it greater freedom to digest the soluble starch.

Sulfanilamide presented a different picture. While ethyl alcohol, ether, and chloroform markedly increased the activity of diastase when they were associated with finely ground charcoal, sulfanilamide did not alter it appreciably. The effect, if any, was a very slight increase in the rate of diastatic activity. This is commensurate with the effect of sulfanilamide

TABLE I

THE EFFECT OF SOME NARCOTICS ON THE DIGESTIVE ACTION OF DIASTASE
IN PRESENCE OF CHARCOAL

NARCOTIC, WITHOUT CHARCOAL	MINIMUM TIME FOR SOLUBLE STARCH SOLUTION TO BE DIGESTED PAST THE LAST IODINE STAINING STAGE
	min.
Control (no narcotic added)	15
Ethyl alcohol, 25 per cent.	30
Ether, 25 per cent.	17
Chloroform, 25 per cent.	25
Sulfanilamide, 0.2 per cent.	19
NARCOTIC, WITH 1 GM. CHARCOAL	
Control (no narcotic added)	234
Ethyl alcohol, 25 per cent.	35
Ether, 25 per cent.	17
Chloroform, 25 per cent.	53
Sulfanilamide, 0.2 per cent.	205

on the action of diastase in the absence of charcoal; *i. e.*, sulfanilamide reduces diastatic activity very slightly as shown by EYSTER (11). The fact that sulfanilamide did not alter, or only slightly increased, the activity of enzymes adsorbed on charcoal may be another part of the reason why sulfa drugs are so effective in controlling bacterial diseases.

These findings are intensely significant because they indicate that diastase in liver cells is adsorbed on cellular colloids such as proteins, and that all enzymes in whatever cell may be similarly associated with colloids. In a study of enzymes it is well to bear this in mind and to make our interpretations accordingly. The influence of a factor on isolated enzymes is not necessarily the same as that on enzymes in cells. The associated colloids dominate the action of enzymes, and factors of the environment may act chiefly in the liberation of enzymes from the associated colloids or vice versa in more completely adsorbing the enzymes on their associated colloidal substances.

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LITERATURE CITED

1. EYSTER, H. C. Enzyme action. Science n. s. **96**: 140–141. 1942.
2. GEIGER, E. Enzyme action. Science n. s. **96**: 426. 1942.
3. JOHNSON, FRANK H., BROWN, DUGALD E. S., and MARSLAND, DOUGLAS A. The mechanism of temperature and hydrostatic pressure reversal of narcosis in luminous bacteria. Anat. Rec. **81**: Supplement, page 33. 1941.

A POSSIBLE NON–ENZYMATIC MECHANISM OF CHANGES OCCURRING IN THE PECTIC SUBSTANCES AND OTHER POLYSACCHARIDES IN LIVING PLANTS[1]

Z. I. KERTESZ

It is usually assumed that enzymes are responsible for most chemical changes occurring in plant tissues. Yet, occasionally such reactions take place without the apparent presence of the corresponding enzymes.

The ripening of many fruits, as apples (4), pears, peaches (1), and tomatoes (2), for instance, is closely associated with progressive transformations of their pectic constituents. These reactions have been commonly assumed to be the results of enzymatic activity. Considerable effort to find the enzymes which cause the hydrolysis of polygalacturonic acid has failed (6), as has a more recent attempt to show their presence in apples (3). The purpose of this note is to suggest a non-enzymatic reaction which is likely to occur in plants and which may be responsible for some of the changes which occur in pectic substances as well as in other polysaccharides.

It has been known for some time that strong oxidizing agents hydrolyze polysaccharides and transform the component sugars into the corresponding dicarboxylic acids. On the other hand, dilute hydrogen peroxide acts on starch to yield dextrins and maltose (5). This observation was recently reiterated by ROBERTSON, ROPES, and BAUER (8) who state that both starch and pectin are degenerated by hydrogen peroxide in the presence of ascorbic acid.

The effect of hydrogen peroxide on the pectic substances and the transformations which are caused will be dealt with elsewhere. Suffice it to say here that dilute solutions of hydrogen peroxide rapidly degenerate dissolved pectin even in the absence of ascorbic acid. The pectin loses its typical colloidal properties, becomes soluble in 70 per cent. alcohol and is transformed into a material which may not be classified any longer as a pectic substance. It is likely that this reaction with hydrogen peroxide is not typical for pectin and starch alone but that it will also be observed with other polysaccharides.

It is suggested that some of the changes in the polysaccharides and pectic constituents of plants *in situ* may be the result of the action of peroxides on these substances. The presence of peroxides in plants has never been conclusively demonstrated. It has been shown, however, that a number of dehydrogenase systems are capable of forming hydrogen peroxide *in vitro*, at least, and that many reactions occur in plants which necessitate the assumption of the presence of peroxides in the tissue. It has been often suggested that hydrogen peroxide or organic peroxides are cell constituents and that their absence, as shown by chemical tests, is only indicative of their transient character.

Now, if peroxides are present in the plant tissue, it may well be that one of their functions is the degeneration and decomposition of starch and pectin. This reaction may also be responsible for the transformation of protopectin into soluble pectin (pectinic acid). It is likely that protopectin is but a pectinic acid of much higher molecular or micellar weight (7). Its transformation into soluble pectin would be essentially the same reaction as the subsequent degradation of pectin (pectinic acid) into d-galacturonic acid. Thus the effect of peroxide may, theoretically at least, replace the function of both protopectinase and pectinase (pectin-polygalacturonase).

If hydrogen peroxide or organic peroxides can be shown to be performing transformations of polysaccharides in plants, this would open up further avenues of speculation concerning the interrelation between oxido-reduction reactions and carbohydrate changes. It is of special interest to note in this connection that hydrogen peroxide apparently occurs among the reaction products during the non-enzymatic decomposition of ascorbic acid (9).

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LITERATURE CITED

1. APPLEMAN, C. O., and CONRAD, C. M. Pectic constituents of peaches and their relation to softening of the fruit. Maryland Agr. Exp. Sta. Bull. 283. 1926.
2. ——————, and ——————. The pectic constituents of tomatoes and their relation to the canned product. Maryland Agr. Exp. Sta. Bull. 291. 1927.
3. BEATTIE, H. G., PEDERSON, C. S., and KERTESZ, Z. I. Unpublished data.
4. BRANFOOT, M. H. (M. H. CARRÉ). A critical and historical study of the pectic substances of plants. (British) Food Investigation Board, Special report no. 33, 1929.
5. GERBER, C. Saccharification of starch by hydrogen peroxide. Compt. Rend. Acad. Sci. (Paris) 154: 1543–1545. 1912. Chem. Abstr. 7: 91. 1913.
6. KERTESZ, Z. I. Pectic enzymes. II. Pectic enzymes of tomatoes. Food Res. 3: 481–487. 1938.
7. ——————. Note on the nature of protopectin and the interrelation of pectic substances. Paper given at the Memphis, Tennessee meeting Amer. Chem. Soc. April 23, 1942. (Unpublished.)
8. ROBERTSON, W. R., ROPES, M. W., and BAUER, WALTER. The degradation of mucins and polysaccharides by ascorbic acid and hydrogen peroxide. Biochem. Jour. 35: 903–908. 1941.
9. STEINMAN, H. G., and DAWSON, C. R. On the mechanism of the ascorbic acid-ascorbic acid oxidase reaction. Jour. Amer. Chem. Soc. 64: 1212–1219. 1942.

AN AIR PUMP OF LOW CAPACITY[1]

Earl B. Working

(WITH TWO FIGURES)

For a study of the respiration and heat production of wheat it was desired to draw a slow stream of air through the grain over long periods of time. Too rapid aspiration greatly increased the difficulty of holding the moisture content of the grain constant, but at slow rates, needle valves or rubber tubing constricted with screw clamps were difficult to adjust accurately and were likely to clog up during the night and void the experiment.
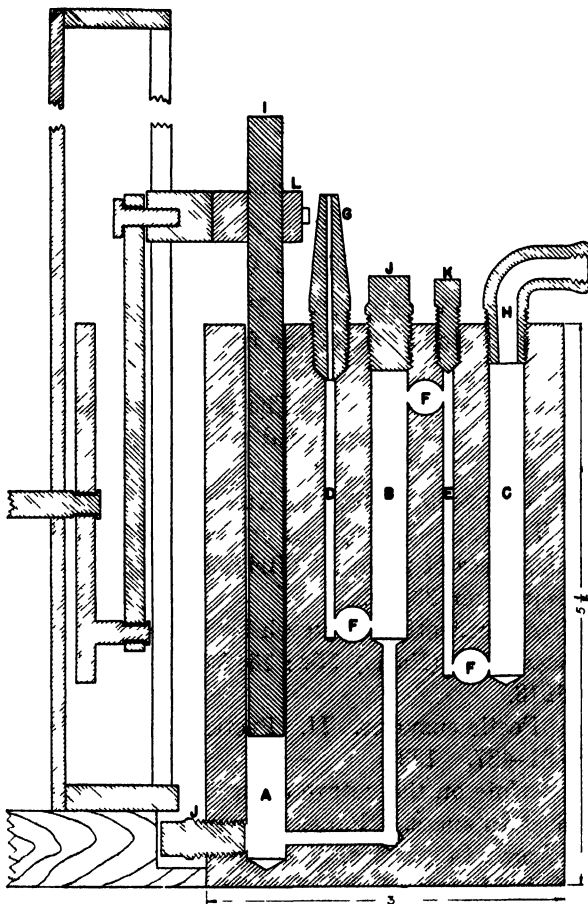
FIG. 1. Air pump drilled from aluminum alloy.

An adjustable pump of low capacity seemed to offer the best solution. It was decided to use mercury valves, since very little leakage or sticking in other types of valves might cause complete failure to pump. It was believed that a mercury piston would remove the possibility of leakage at that point,

In the lubrication of motorcycle motors the principle has been used that the output of a pump can be varied continuously from zero to maximum without change of the rate or length of stroke, if the position of the piston is adjustable so that any desired portion of the stroke may occur before the piston reaches the inlet port, and the inlet valve consists only of the closing of the inlet port by the passage of the piston. This principle of adjustment is especially easy to apply to a mercury piston.
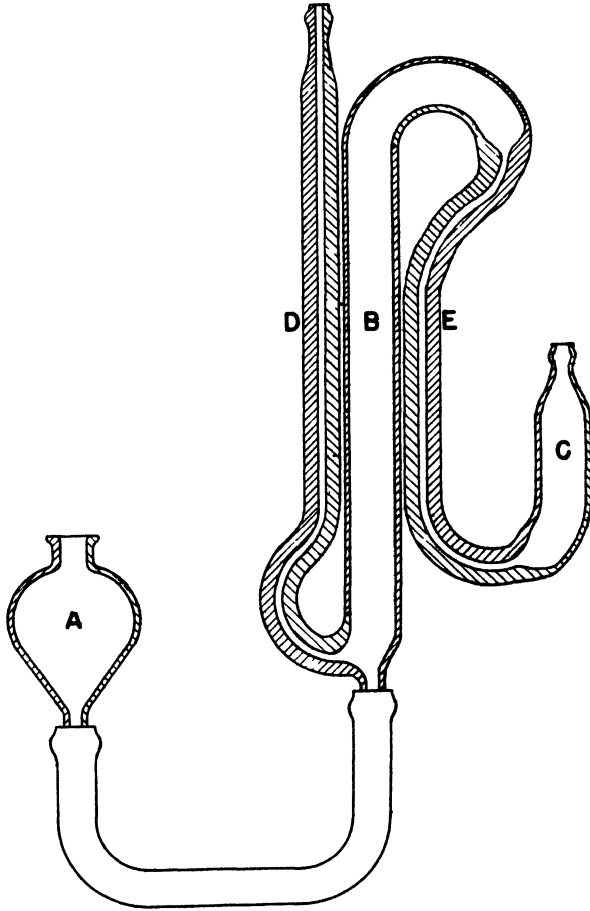
Fig. 2. Air pump constructed of glass and rubber tubing.

The first of these pumps was drilled out of a bar of hard aluminum alloy, $1 \times 3 \times 5\frac{1}{2}$ inches, as shown in figure 1, because it was to be used in a location where there was serious danger of breakage of a glass pump. The connections, F, F, F, between the vertical bores, D, B, E, and C, were made by drilling in from the side, and closing the holes with $\frac{1}{8}$-inch iron pipe plugs, such as were also used at J, J. Because of space limitations, a special plug, K, was made from $\frac{1}{4}$-inch rod to close the upper end of bore E.

For operation, bore A is filled with mercury to a level immediately below the opening of B into D, and barely enough mercury is put into C to fill bore

E when there is sufficient suction on that side. The steel plunger, I, is adjustable vertically in clamp L so that at the bottom of its stroke it may displace any amount from zero to nearly all of the mercury in the reservoir A. As this mercury is displaced, it rises in B, first closing the inlet valve from D, and then forming the piston of the pump, forcing air from B through the small amount of mercury forming a seal at the bottom of C. On the return stroke, however, this seal will withstand a suction of about $2\frac{1}{2}$ inches of mercury because of the small volume of bore E. Thus the suction or pressure obtainable from such a pump is limited chiefly by the length of the small diameter drill available to cut D and E.

Ordinarily it will be much easier to construct the pump of glass and rubber tubing as shown in figure 2. This also obviates the necessity of using guides and a connecting rod to get straight line reciprocating motion, since the reservoir A may simply be hung from a slowly-revolving crank. The output of the pump can then be varied from maximum to zero by raising the body of the pump to reduce the effective portion of the stroke. The dimensions of the pump may be chosen to suit individual requirements. A bore diameter of from 1 to 2 millimeters is suggested for D and E, and of from 10 to 20 millimeters for B and C. The suction or pressure obtainable is limited by the vertical height of the mercury column in E, and 10 to 15 centimeters is suggested as sufficient in most cases. If the pump is operated against a pressure greater than this, as would occur if the inlet or exhaust were left closed off, the mercury may be blown out of capillary E, so the tube B is bent over at the top to form a trap so that the mercury will run back down into E when pressures are restored to normal. The pump would be slightly more efficient if the bend were made in the capillary E, so that the mercury piston could rise to the top of the larger diameter tube B, in which case it would be desirable to place a small trap near the top of E to prevent the loss of the mercury which forms the valve.

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NOTES

Sectional News.—The editor wishes to remind the chairmen and secretaries of all sections, both regional and local, that PLANT PHYSIOLOGY desires to disseminate sectional news to the membership at large. It is necessary, however, that the news be sent in voluntarily, and in time for appropriate use. Announcements must be sent in long enough before the events to have them reach the members ahead of the events. Spring meetings cannot be announced any later than the April number; and copy must be in hand some weeks before the journal appears, or it cannot be used. Reports of meetings are desirable, but should be sent in promptly after the meetings, so that the reports are timely. It is not possible to write to the officers of sections for such information, and the journal will carry only such notices as are sent in early enough to meet the suggested timing.

Manuscripts.—It seems desirable once more to call attention to the machinery set up for the handling of manuscripts offered to PLANT PHYSIOLOGY. The editor-in-chief no longer circulates papers to the readers. That function is performed by the secretary of the editorial committee, Dr. WALTER F. LOEHWING, Department of Botany, The University of Iowa, Iowa City, Iowa. When papers are sent direct to Chicago, they do not receive attention for some weeks. There is no time for the readdressing and reshipment of such manuscripts. They accumulate in a drawer until they get in the road; then they may receive attention, when a single trip will care for numbers of them. If authors wish prompt action on their papers, they can get it, by following the rules which are published on the third cover page of every number of the official journal. There is enough work on the actual editorial work that we cannot spend time on the manuscripts which are entered through improper channels. We want to be helpful, prompt, courteous to all; but we need the cooperation of all writers who desire to publish in PLANT PHYSIOLOGY. Your journals can reach you more promptly if we do not have so many extraneous errands to run.

Membership List.—The secretary is preparing a new membership list. It has always seemed to appear at the wrong time of year, so the editor wishes to make a suggestion. There is one period when the membership list is necessary to intelligent action; that is the period of nominations and election of officers. When the list appears after that period is past, it is out of date by the time another election comes round. Would it not be much better to prepare the membership list during the autumn, or late summer, and send it out either with the October or January number of PLANT PHYSIOLOGY? It would then be in hand for the intelligent selection of nominees, and the mailing with the journal should save some postage. Some other societies publish

their roster of members with their annual year-books. We could add the membership list following the index in the October number of PLANT PHYSIOLOGY, and so provide a solution to a problem which has long wanted intelligent action.

Enzymology.—The third volume of this series, *Advances in Enzymology and Related Subjects of Biochemistry*, has been published by Interscience Publishers, Inc., 215 Fourth Ave., New York City. The series is edited by F. F. NORD, of Fordham University, and C. H. WERKMAN, of Iowa State College. All plant physiologists will be grateful that it bears the portrait of RICHARD WILLSTÄTTER as a frontispiece, in memory of this great biochemist who died in 1942 at the age of 70. There are eleven summaries of progress in the field of enzyme chemistry. These are entitled as follows: Chromosomes and nucleoproteins, by A. E. MIRSKY; effects of temperature on enzyme kinetics, by IRWIN W. SIZER; x-rays and the stoichiometry of the proteins, by W. T. ASBURY; the chemistry of glycogen, by KURT H. MEYER; verdoperoxidase, by KJELL AGNER; mechanisms of carbohydrate metabolism, by E. S. GUZMAN BARRON; the intermediary stages in the biological oxidation of carbohydrate, by H. A. KREBS; the chemistry and biochemistry of pantothenic acid, by ROGER J. WILLIAMS; the chemistry and biochemistry of biotin, by KLAUS HOFMANN; recent progress in tumor enzymology, by JESSE P. GREENSTEIN; and the rôle of microorganisms and enzymes in wine making, by W. V. CRUESS.

The standing of the various writers in their field guarantees that the summaries of progress are well considered reports of the work recently accomplished in enzymology and closely related biochemical research. In addition, this volume has not only an author and subject index, but it contains a cumulative index for the three volumes which have thus far appeared in the enzymology series. This cumulative index is very short, but lists the main things, authors, and subjects in broad terms, so that all three volumes may be consulted quickly for any contributor's review, and for the general subject. It makes use very easy.

The publishers, contributors, and the editors deserve our thanks and congratulations that so much service can be rendered to their fellow workers during these troublous times. The price is only $5.50 per copy, which easily puts it in reach of all workers and libraries. It is highly commended, and will no doubt receive a hearty welcome from all workers in this field of investigation.

FOOD RESERVE DEPLETION AND SYNTHESIS IN FIELD BIND-
WEED, *CONVOLVULUS ARVENSIS* L., AS RELATED TO
7–DAY AND 14–DAY INTERVALS OF
CULTIVATION[1]

JOHN C. FRAZIER

Introduction

A study has been made of regenerated bindweed plants which developed
following cultivation to a depth of 4 inches. It dealt primarily with the
relative depletion of reserve foods by such a cultivation at 7-day and 14-day
intervals as determined on plants grown under light-proof covers. Secon-
darily it sought to obtain information indicating the extent of synthesis of
readily available carbohydrates and protein nitrogen in plants in light dur-
ing the first 8 days following emergence after such a cultivation. The term
plant as used in this and in a previous study (4) includes the 4-inch rhizome
(underground stem) which arises from each root severed at that depth by
means of a cultivating instrument and the shoot, or shoots, borne on the
same.

A previous paper (4), based on the plant material used in this study,
showed that on a dry weight basis such bindweed plants, grown in the field
under light-proof covers for 8 days after shoot emergence, had shoots that
weighed twice as much as the 4 inches of rhizome, the development of which
brought about emergence. Since all development of the covered plants
must have come from stored food, it appeared logical to conclude that twice
the amount of food reserves is required to form the shoots as to form the 4
inches of rhizome. Other workers (3, 9) have demonstrated that reserve
food depletion in the underground parts of field bindweed grown in full
light in the field is continued for at least 8 days after emergence following
cultivation. It would appear, therefore, that such plants continue to draw
upon the food reserves in the underground parts for this period.

This paper reports findings on the material used in an earlier study (4)
in terms of the following components: Reducing sugars, total sugars, the
starch-dextrin fraction, the computed readily available carbohydrate frac-
tion, and the total nitrogen, protein nitrogen, and the computed "true"
protein fractions.

Material and methods

The dates on which the material was secured and its previous cultivation treatment are given in table I. Three areas within 8 miles of Manhattan having a loam soil type were used. Area A was a heavy loam, B, light, and C, intermediate in this respect. The precipitation data were given in a previous paper (4). The method of securing this material is repeated here. Typical bindweed shoots growing normally in the field were selected as they emerged 6 days after cultivation (to a depth of 4 inches) and immediately covered with an earthen jar of 1- to 2-gallon capacity. The same number

TABLE I

SUMMARY OF FIELD DATA ON REGENERATED PLANTS OF *Convolvulus arvensis*. MANHATTAN, KANSAS, 1940–1941

AREA	DATE	NUMBER OF CULTIVATIONS 1939	NUMBER OF CULTIVATIONS 1940	NUMBER OF PLANTS	COVERED 4-IN. SECTION OF RHIZOME	COVERED SHOOTS	COVERED RATIO	EXPOSED 4-IN. SECTION OF RHIZOME	EXPOSED SHOOTS	EXPOSED RATIO
	1940				*gm.*	*gm.*		*gm.*	*gm.*	
A	May 3–11	None	None	50	1.133	3.511		0.945	7.142	
B	Aug. 12–20	12	6	40	0.816	1.062		0.784	2.330	
A	Aug. 20–28	None	7	35	0.646	1.407		0.638	3.966	
B	Aug. 30–Sept. 7	12	7	50	0.590	1.308		0.588	2.151	
A	Oct. 5–14	None	10	35	0.301	0.575		0.324	1.216	
B	Oct. 28–Nov. 5	12	10	35	0.414	0.580		0.417	0.750	
				245	3.900	8.443	2.16	3.696	17.555	4.75
	1941	1940	1941							
C	April 14–22	6*	0	35	0.885	1.656		0.923	3.526	
C	June 27–July 5	6*	5	45	0.741	2.496		1.065	7.407	
C	July 11–19	6*	6	65	1.409	4.424		2.285	15.433	
				145	3.035	8.580	2.83	4.273	26.366	6.17

* July 24 to Nov. 4, 1940.

of similar shoots was selected and marked at the same time but was not covered. Eight days later both sets were harvested by severing the shoots at the ground and excavating the 4 inches of rhizome, the development of which had preceded emergence. Thus 4 inches of rhizome (underground part of the stem) and all the leafy shoot (aboveground stem with its leaves) were obtained. The shoots and 4-inch lengths of rhizomes of 245 covered plants and an equal number of plants exposed to sunlight were collected in 1940, and 145 from each situation in 1941.

All plant material secured was killed by holding in a forced circulation electric oven at 105° C. for 20 minutes, following which it was dried 14 hours at 70° C. It was coarsely ground, reduced to 60-mesh size by means of a mortar and pestle and stored in sealed glass containers until analyzed. At

TABLE II

SUMMARY OF ANALYTICAL DATA (ON DRY WEIGHT BASIS) SHOWING THE CONSTITUENT RESERVES IN REGENERATED PLANTS OF *Convolvulus arvensis*. MANHATTAN, KANSAS, 1940–1941

MATERIAL ANALYZED	NUMBER OF PLANTS	OVEN-DRY WT. OF SHOOT OR 4-IN. SECTION OF RHIZOME (gm.)	REDUCING SUGAR (%)	TOTAL SUGARS (%)	STARCH-DEXTRIN FRACTION (%)	READILY AVAILABLE CARBOHYDRATE FRACTION (%)	READILY AVAILABLE CARBOHYDRATE FRACTION (mg.)	TOTAL NITROGEN (%)	TOTAL NITROGEN (mg.)	PROTEIN NITROGEN (%)	PROTEIN NITROGEN (mg.)	"TRUE" PROTEIN (PROTEIN N × 6.25) (mg.)
1940 Covered plants	245											
Rhizomes		3.898	1.89	6.06	4.75	10.81	421.4	3.71	144.6	2.95	115.0	718.8
Shoots		8.444	0.82	2.30	3.98	6.28	530.3	5.01	423.0	4.17	352.1	2200.6
Ratio		2.17					1.26				3.06	
Exposed plants	245											
Rhizomes		3.696	2.30	5.42	4.28	9.70	358.5	3.64	134.5	2.89	106.8	667.5
Shoots		17.554	0.64	3.54	6.31	9.85	1729.1	4.88	856.6	3.97	696.9	4355.6
Ratio		4.75					4.82				6.53	
1941 Covered plants	145											
Rhizomes		3.034	1.80	6.01	4.44	10.45	317.1	3.79	115.0	3.01	91.3	570.6
Shoots		8.577	1.07	2.64	3.88	6.52	559.2	5.09	436.6	4.19	359.4	2246.3
Ratio		2.83					1.76				3.94	
Exposed plants	145											
Rhizomes		4.273	1.80	6.09	4.41	10.50	448.7	3.58	153.0	2.84	121.4	758.8
Shoots		26.365	0.33	3.32	6.44	9.76	2573.2	4.71	1241.8	3.90	1028.2	6426.3
Ratio		6.17					5.73				8.47	

this time it was redried at 105° C., and 2-gram samples for the carbohydrate studies and 0.5-gram samples for the nitrogen studies were weighed out.

The carbohydrate analyses were made essentially as reported by FRAZIER (5) except that a 2-gram sample was used and the starch-dextrin fraction was hydrolyzed for an hour at 120° C. in an autoclave. The total nitrogen and protein nitrogen analyses were made as reported by MILLER (8). These did not include inorganic nitrogen, since the diphenylamine test showed this to be unnecessary. The "true" protein component was obtained by multiplying the protein nitrogen component by the factor 6.25.

Results

The analytical results are given in table II. These are reported as percentages (on a dry weight basis), or milligrams, or both, of the reducing

TABLE III

READILY AVAILABLE CARBOHYDRATES AND PROTEIN NITROGEN TH\T WOULD BE WITHDRAWN FROM THE UNDERGROUND PARTS OF COVERED BINDWEED PLANTS WITH 1 CULTIVATION AT THE END OF 14 DAYS COMPARED WITH 2 CULTIVATIONS AT 7 DAY INTERVALS. MANHATTAN, KANSAS, 1940–1941

CULTURE	1940 245 PLANTS		1941 145 PLANTS	
	READILY AVAILABLE CARBO HYDRATES	PROTEIN NITROGEN	READILY AVAILABLE CARBO HYDRATES	PROTEIN NITROGEN
2 cultivations, i.e., two sets of rhizomes destroyed*	843 mg.	230 mg.	634 mg.	183 mg.
1 cultivation, i.e., one set of rhizomes + 1 set of shoots destroyed	952 mg.	467 mg.	876 mg.	451 mg.
Ratio	1.13	2.03	1.38	2.46
Excess of withdrawal in favor of 1 cultivation	13%	103%	38%	146%

* Cultivation every 7th day permitted no shoot development.

sugars, total sugars, starch-dextrins, readily available carbohydrates, total nitrogen, protein nitrogen and "true" protein in the known weight of a known number of rhizomes and shoots grown under cover and an equal number grown in full light.

In 1940 the shoots of the covered plants contained 1.26 times as much readily available carbohydrates and 3.06 times as much protein nitrogen as the rhizomes of those plants. In 1941 the corresponding ratios were 1.76 and 3.94. For the exposed plants these ratios were 4.82 and 6.53 in 1940 and 5.73 and 8.47 in 1941, respectively.

The relative depletion of food reserves (carbohydrates and nitrogen), supplied by the underground parts of the plant, by cultivation every 7th day as compared with cultivation every 14th day, was calculated for the covered plants. These calculations were made by adding the weight of the readily available carbohydrates or the protein nitrogen found in the rhizome

to the weight of that component found in the shoot (since both would be destroyed by cultivation on the 14th day) and comparing the sum with twice the weight of that found in the rhizome, as two sets of rhizomes, but no shoots, would be destroyed by cultivating at 7-day intervals. The data obtained by these calculations are given in table III. The depletion of readily available carbohydrates was 13 per cent. greater in 1940, and 38 per cent. greater in 1941; and for protein nitrogen the depletion was 103 per cent. greater in 1940, and 146 per cent. greater in 1941 when the one culti-

TABLE IV

COMPARISON OF READILY AVAILABLE CARBOHYDRATES, PROTEIN NITROGEN, AND ''TRUE'' PROTEIN IN THE RHIZOMES AND SHOOTS OF PLANTS COVERED AND IN LIGHT. MANHATTAN, KANSAS, 1940–1941

MATERIAL ANALYZED	1940 245 PLANTS			1941 145 PLANTS		
	READILY AVAILABLE CARBO HYDRATES	PROTEIN NITROGEN	''TRUE'' PROTEIN	READILY AVAILABLF CARBO HYDRATES	PROTEIN NITROGEN	''TRUE'' PROTEIN
	mg.	mg.	mg.	mg.	mg.	mg.
Rhizomes						
Exposed plants	359	107	668	449	121	759
Covered plants	421	115	719	317	91	571
Difference	− 62	− 8	− 51	132	30	188
Difference per plant	− 0.25	− 0.03	− 0.21	0.91	0.21	1.30
Shoots						
Exposed plants	1729	697	4356	2573	1028	6426
Covered plants	530	352	2201	559	359	2246
Difference	1199	345	2155	2014	669	4180
Difference per plant	4.89	1.41	8.80	13.89	4.61	28.83
Total difference per plant (rhizome plus shoots)	4.64	1.38	8.59	14.80	4.82	30.13

vation was made on the 14th day. In addition such a procedure involved but half the labor.

Based upon the assumption that the rate and extent of reserves supplied by the underground parts were the same for plants in light as for covered plants, calculations were made to compare the average gain in readily available carbohydrates and protein nitrogen for the shoots and rhizomes during the 8-day period following emergence. These data are given in table IV. They show that the average total increase of readily available carbohydrates in the plant (rhizome plus shoots), in light as compared with those covered, for this 8-day period was 4.64 mg. in the 245 plants studied in 1940, and 14.80 mg. in the 145 plants in the 1941 studies. These amounts apparently represent the average synthesis of this fraction per plant during this 8-day interval. This amounts to an average daily gain of 0.6 mg. in the plants studied in 1940, and 1.8 mg. in those studied in 1941; however, this average daily gain is given for comparative purposes only, as probably most of it

accumulated the last 2 or 3 days of this 8-day period, *i.e.*, after the shoot had established its green surfaces.

The total nitrogen accumulation represents inorganic nitrogen which had been utilized in the formation of organic nitrogenous compounds, particularly proteins, plus that which entered the plant parts studied because of the inorganic nitrogen in those parts being so utilized.

The data of table IV show that the average total increase of protein nitrogen in the rhizome plus shoot, in light, for this 8-day period was 1.38 mg. in the 245 plants used in 1940, and 4.82 mg. in the 145 plants studied in 1941. The calculated average daily increase per plant was 0.17 mg. in 1940, and 0.6 mg. in 1941.

Another possible explanation of the greater amount of reserves in plants growing in light is that part or all was obtained from root reserves. Additional studies are contemplated in which a 4- to 6-inch section of the roots which bear the rhizomes will be obtained in addition to the rhizomes and shoots of plants growing in light and under light-proof covers. If no appreciable difference is found in the reserve content of these roots, definite evidence will have been obtained that the difference in reserves in rhizomes plus shoots growing in light and under light-proof covers is the result of photosynthesis occurring in those in light.

Discussion

Successful control of noxious perennial weeds has long been based upon the suppression of shoot growth in order that the underground parts be depleted of their food reserves. In recent years it has been recognized that shoots may emerge and grow for several days before returning food reserves to the underground parts (3, 7, 9, 11). TIMMONS (10) reported that in most cases the decrease in reserves was more rapid for several days after than prior to emergence of the bindweed following cultivation.

The 8-day interval following emergence, used in this study, added to the 6 days commonly required under average growing conditions to bring about emergence, when cultivations are made to a depth of 4 inches, gives the 14-day cultivation interval recommended for bindweed control in Kansas (6).

The term "readily available carbohydrates" as commonly understood includes the total sugars and the starch-dextrin fraction. Recent work (1, 3, 10, 11) indicates that this composite fraction is the most meaningful one in the carbohydrate economy of the plant. It is generally agreed that all portions of this fraction (freed of gums by clearing) are readily utilized as food reserves by the plant if, as BAKKE *et al.* (1) stated, depletion can be taken as a measure of utilization.

The differences noted in the food reserve relations between the two growing seasons is believed due in part to the more vigorous shoot growth found in the patch studied in 1941 plus the fact that two of the three studies made that year were at the time of marked growth, while only one of the six studies made in 1940 was during the more vigorous early spring growth.

Possibly the rhizomes secured in this experiment were different in their food reserve relations than had they been taken at the time of their emergence. To ascertain if such is the case, 25 rhizomes were taken at each of the 3 periods (table I) of sampling in 1941 or a total of 75. These weighed 1.209 gm. of which 144.96 mg., or 11.99 per cent., consisted of readily available carbohydrates. This is an average of 1.93 mg. per rhizome. This does not seem significantly different from the 1.89 mg. average weight found in the 390 rhizomes (1940–1941) grown under cover which is the group with which this comparison should be made. No comparable study was made for the nitrogenous fractions.

The percentages of the carbohydrate fractions may appear low but the bindweed being studied was subjected to cultivation at 14-day intervals. They are in general agreement with findings on bindweed subjected to such treatment in Colorado (**2**). The specific cultivation treatment given the plants reported in this study is to be found in table I.

Studies on the nitrogenous fractions have been reported by other workers (**1, 3, 7, 10**). There is general agreement that the rôle played by these fractions is not well understood at present. BAKKE *et al.* (**1**) reported that both colloidal and noncolloidal nitrogen were reduced in plants grown on fallowed land. BARR (**3**), however, found that the soluble-nitrogen content remained about the same in either cultivated or undisturbed bindweed roots, while cultivation reduced the colloidal- or protein-nitrogen content, although frequency of cultivation appeared to have no material effect. These investigators (**1, 3**) used a different reagent for fractionation, hence no direct comparison can be made with the work reported herein. BARR (**3**) reasoned that such a shift with the soluble nitrogen materials going into the developing shoot would account for the rapid reduction he observed in protein nitrogen after cultivation, a reduction which continued for at least 10 to 15 days after cultivation.

The present study indicates that there is a rapid accumulation of nitrogenous materials in the rhizomes and shoots once they start developing and that a large proportion, approximately 80 per cent., is present as protein nitrogen. Because of the method of calculation the trends of "true" proteins paralleled those obtained for protein nitrogen.

Summary

1. Regenerated bindweed plants which developed following cultivation to a depth of 4 inches were studied. These consisted of the 4-inch section of rhizome which arose from roots severed at that depth and the shoot, or shoots, borne on that rhizome.

2. Comparable numbers of such regenerated plants were studied after 8 days of shoot development under normal sunlight and under light-proof covers.

3. The material was assembled into four composite samples, *viz.*, rhizomes exposed, shoots exposed, rhizomes covered, and shoots covered, for each year

of the study. These were analyzed for various carbohydrate and nitrogen fractions. The data are reported as percentages (on a dry-weight basis) or milligrams, or both, of the reducing sugars, total sugars, starch dextrins, readily available carbohydrates, total nitrogen, protein nitrogen, and "true" protein.

4. The relative amounts of the two fractions (the readily available carbohydrates and the protein nitrogen) in the two parts, rhizomes and shoots of plants grown under each light condition, covered and exposed, were determined for the two growing seasons.

5. The relative depletion of food reserves, supplied by the underground parts of the plant, by cultivation every 7th day as compared with cultivation every 14th day, was calculated. From this it appears that cultivation every 14 days would destroy a fifth more of the readily available carbohydrates and more than double the loss of protein nitrogen in the two parts as compared with two cultivations at intervals of 7 days in the same unit of time.

6. Synthesis by the plants growing in light is considered to account for the higher reserves of those plants. Based upon the assumption that the rate and extent of reserves supplied by the underground parts were the same for plants in light as for covered plants, data are presented showing the average gain per plant of readily available carbohydrates and of protein nitrogen for the 8-day period of shoot development following emergence and the average gain of these fractions per plant per day for the two growing seasons.

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LITERATURE CITED

1. BAKKE, A. L., GAESSLER, W. G.. and LOOMIS, W. E. Relation of root reserves to control of European bindweed, *Convolvulus arvensis* L. Iowa Agr. Exp. Sta. Res. Bull. 254. 1939.
2. BARR, C. GUINN. Preliminary studies on the carbohydrates in the roots of bindweed. Jour. Amer. Soc. Agron. **28**: 787–798. 1936.
3. ——————. Organic reserves in the roots of bindweed. Jour. Agr. Res. **60**: 391–413. 1940.
4. FRAZIER, JOHN C. Influence of time of cultivation on the control of field bindweed, *Convolvulus arvensis* L. Trans. Kansas Acad. Sci. **44**: 164–169. 1941.
5. ——————. Amount, distribution and seasonal trend of certain organic reserves in the root system of field bindweed, *Convolvulus arvensis* L. Plant Physiol. **18**: 167–184. 1943.
6. The bindweed program of Kansas. Report of the State Board of Agriculture. Vol. **57**, no. 227. 42 pp. 1938.
7. KIESSELBACH, T. A., PETERSEN, N. F., and BURR, W. W. Bindweeds and their control. Nebraska Agr. Exp. Sta. Bull. 287. 1934.

8. MILLER, E. C.   A physiological study of the winter wheat plant at different stages of its development.   Kansas Agr. Exp. Sta. Tech. Bull. 47.   1939.

9. TIMMONS, F. L.   Annual report from the bindweed control project. Fort Hays (Kansas) Branch Exp. Sta.   1937.   Unpublished. (pp. 43 and 51 cited.)

10. —————.   Annual report from the bindweed control project. Fort Hays (Kansas) Branch Exp. Sta. 1939.   Unpublished.   (p. 42 cited.)

11. —————.   Results of bindweed control experiments at the Fort Hays Branch Station, Hays, Kansas, 1935 to 1940.   Kansas Agr. Exp. Sta. Bull. 296.   1941.

NUTRIENT DEFICIENCIES IN THE STRAWBERRY LEAF AND FRUIT[1]

R. A. Lineberry and Leland Burkhart

(WITH SEVEN FIGURES)

Introduction

Previous investigational work on the nutrition on the strawberry has been concerned largely with the relationship of soil and fertilizer treatment to growth and yield (5). While these studies have increased greatly the knowledge of the fertilizer requirements of the strawberry, they did not describe the symptoms of mineral deficiencies nor show the mineral composition of the plant and fruit. It would appear that foliar symptoms and mineral composition of the plant might be correlated with nutritional needs. In this connection the most notable contribution has been made by Davis, Hill, and Johnson (4). They found that foliar symptoms could be correlated with nutritional needs and that certain positive and negative correlations existed between pairs of elements in the ash of the plant. These relationships were established for the Parson Beauty variety grown under outdoor conditions in Canada. Water color illustrations of potassium-, phosphate-, and nitrogen-deficient strawberry leaves (variety not mentioned) have been published by Davis and Hill (3). Hoagland and Snyder (6) have reported on the effects of potassium, phosphate, chloride, and boron on the growth of the strawberry plant in water cultures in California.

The effect of certain mineral deficiencies on leaf characteristics and associated mineral composition of the foliage and fruit of the strawberry is reported herein. The Klondike and the Blakemore were used as these are the two leading commercial varieties grown in North Carolina.

Materials and methods

In this study, dormant plants of Blakemore and Klondike, with fruit buds well formed, were removed from uniformly fertilized fields to the greenhouse early in January. The roots were washed free from soil, the old leaves were removed, and the plants were set in washed quartz sand in two-gallon glazed earthenware containers which were provided with drainage. Two plants were set in each container and twenty plants of each variety were included in each treatment. The mineral content of dormant field-grown Klondike strawberry plants was determined at the beginning of the experiment. The composition of the respective nutrient solutions employed is shown in table I. These solutions were buffered at pH 5.6 and were applied daily, using the sand-culture technique. Preliminary studies of the

growth of strawberry plants in sand cultures indicated that the presence of chlorides in the nutrient solutions was objectionable except at very low concentrations. High concentrations of sulphate ions also proved objectionable. The chloride ion was, therefore, omitted from all nutrient solutions and sulphate concentrations were kept low (table I).

TABLE I

COMPOSITION OF NUTRIENT SOLUTIONS*

SERIES	PARTIAL VOLUME MOLECULAR CONCENTRATION OF SALTS									
	$Ca(NO_3)_2$	KH_2PO_4	$MgSO_4$	$CaSO_4$	NaH_2PO_4	$NaNO_3$	Na_2SO_4	K_2SO_4	NH_4NO_3	$Mg(NO_3)_2$
Complete	0.004	0.002	0.002						0.001	
Minus–Ca		0.002	0.002			0.008			0.001	
Minus–K	0.004		0.002		0.002				0.001	
Minus–Mg	0.004	0.002					0.002		0.001	
Minus–P	0.004		0.002					0.007	0.001	
Minus–S	0.004	0.002							0.001	0.002
Minus–N		0.002	0.002	0.004						

* Boron at the rate of 0.5 p.p.m. and manganese, iron, zinc, and copper at the rate of 0.25 p.p.m. were added to all nutrient solutions.

The studies were made during the fruiting stage of development in January and February. Mature leaves showing deficiency symptoms were sampled for chemical analysis and ripe fruits from the plants receiving the various treatments were sampled at the time the leaf deficiency symptoms appeared. The fresh leaves and fruit were extracted and analyzed by the procedure described by BURKHART and PAGE (1).

Results

NUTRIENTS IN FOLIAGE AND FRUIT

At the beginning of the experiment, samples of dormant field-grown Klondike plants were taken for the determination of the soluble minerals present in the leaf blades, petioles, crowns, and roots (table II). In the dormant state, there was a considerable accumulation of potassium in the roots as compared with that in leaves and crowns Soluble calcium, mag-

TABLE II

DISTRIBUTION OF SOLUBLE MINERALS IN DORMANT KLONDIKE STRAWBERRY PLANTS, CONSTITUENTS EXPRESSED AS P.P.M. OF THE FRESH TISSUE

	Ca	K	Mg	PO_4	SO_4
	p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.
Leaf blades	2325	2750	2625	5000	400
Petioles	550	3750	2000	1500	250
Crowns	200	2250	1000	2000	2500
Roots	1100	4000	1500	1500	2000

nesium, and phosphate were somewhat localized in the leaf blades. Petioles were low in soluble calcium and sulphate and the crowns were relatively very low in soluble calcium.

After growing in sand culture for about two months, until they fruited and deficiency symptoms were apparent, determinations were made of the soluble-nutrient levels in both leaves and fruit of the Blakemore variety, with the results shown in figures 1 and 2. The potassium concentrations of
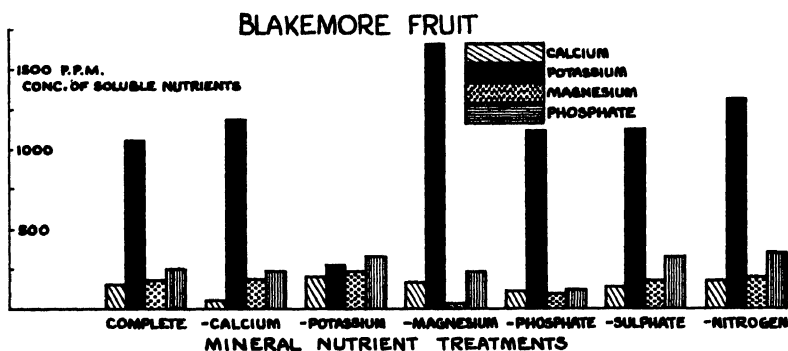
Fig. 1. Soluble nutrients (expressed as parts per million in foliage and fruit) found in Blakemore strawberry plants, grown in nutrient solutions with the indicated deficiencies in certain elements, and in complete nutrient solution.
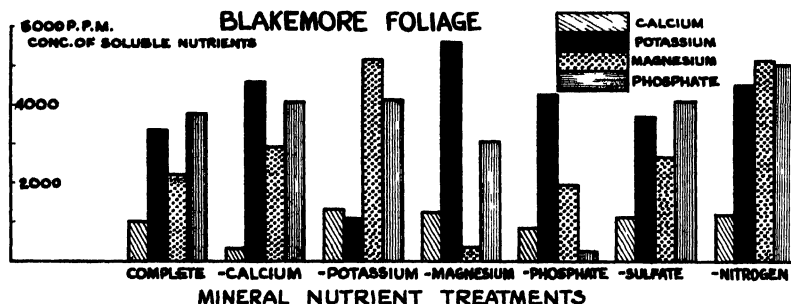
Fig. 2. Soluble nutrients (expressed as parts per million in foliage and fruit) found in Blakemore strawberry plants, grown in nutrient solutions with the indicated deficiencies in certain elements, and in complete nutrient solution.

both leaves and fruit were greatly affected by the ionic nature of the nutrient. Lack of calcium, and especially of magnesium, resulted in a marked increase in potassium in the foliage and the fruit as compared with the effects of the complete nutrient treatment. Plants grown in the potassium-deficient nutrient solution produced foliage containing 1200 p.p.m. of potassium and fruit with 400 p.p.m. of this element, each value being about one-third of that for plants in the complete nutrient series. In the complete nutrient treatment the potassium concentration in relation to the other three soluble minerals was much less in the fruit than in the leaves. There was practically no soluble phosphate in either foliage or fruit at the time phosphorus deficiency symptoms were evident.
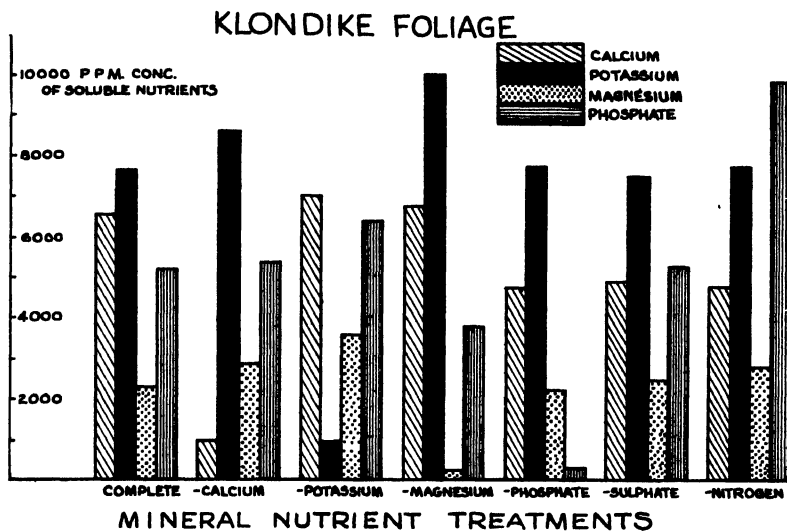
FIG. 3.   Soluble nutrients found in Klondike strawberry plants grown in solutions with the indicated deficiencies in certain elements and in complete nutrient solution.   Expressed in parts per million of fresh foliage and fruit.

In the Klondike variety (figs. 3 and 4) the concentration of soluble potassium and calcium in both leaves and fruit of plants that received complete nutrient solution were much higher than in the Blakemore variety. The ionic antagonistic effects of potassium and magnesium upon the concentrations of their ions in the foliage and fruit of Klondike were very much the same as in the Blakemore.   The relatively high potassium concentration in the fruit of Klondike in relation to other soluble minerals was also evi-
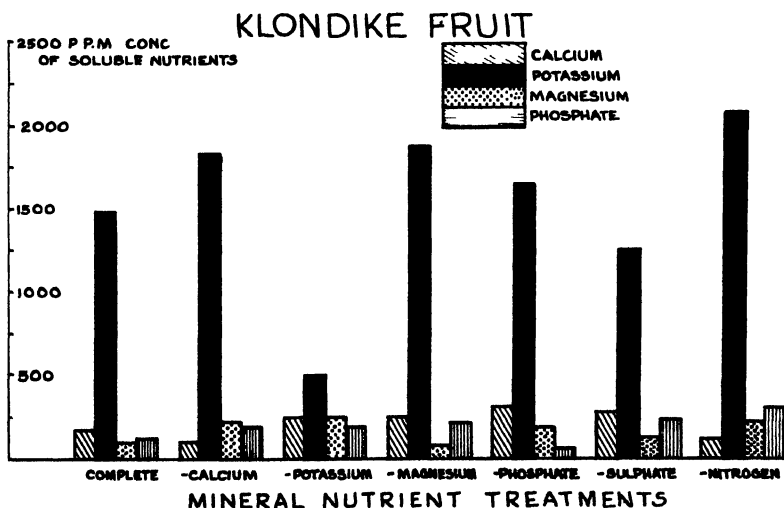
FIG. 4.   Soluble nutrients found in Klondike strawberry plants grown in solutions with the indicated deficiencies in certain elements and in complete nutrient solution. Expressed in parts per million of fresh foliage and fruit.

dent. Soluble phosphate also accumulated in potassium-deficient foliage and fruit. The accumulation of soluble phosphate was very marked in nitrogen-deficient leaves. In the fruit, potassium accumulation in high degree was associated with nitrogen deficiency.

DAVIS *et al.* (4) also found negative correlations in the leaf ash of the Parson Beauty variety of K_2O with CaO, MgO, P_2O_5, respectively, but a positive correlation of MgO with P_2O_5. WALLACE (8) has reported a high nitrogen content of strawberry fruit when potassium was deficient in the nutrient medium.

<div align="center">DEFICIENCY SYMPTOMS</div>

POTASSIUM DEFICIENCY.—A distinct varietal difference was noted in the potassium deficiency symptoms on the leaves. In the Klondike variety, potassium deficiency was characterized by progressive necrosis of the petioles just below the leaflets as shown in figure 5, B and C. Concurrently the mid-
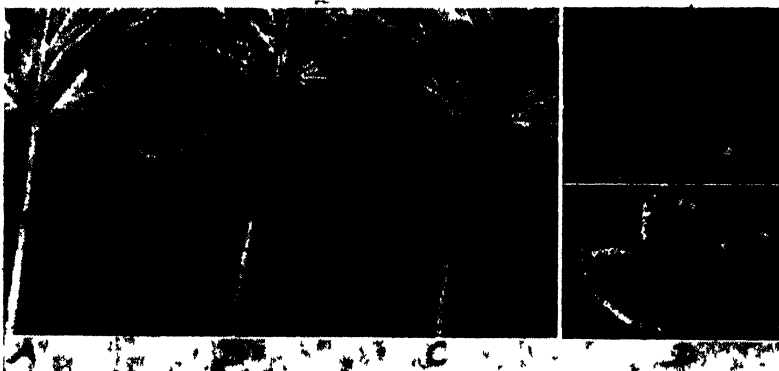
FIG. 5. Potassium deficiency·symptoms in strawberry leaves. A, healthy leaf of the Klondike variety; B and C, progressive necrosis in petioles of this variety; D, marginal leaf scorch in the Blakemore variety.

ribs of the leaflets became purple. As a result of the breakdown of the conducting tissue in the petioles the leaflets wilted and soon dried up. In the Blakemore variety, though analysis showed the potassium level to be about the same as in the Klondike, this type of injury was not observed. In the Blakemore there was a marginal necrosis of the leaflets which gradually rolled upward and inward (fig. 5, D) and the younger leaflets became light green to yellow between the veins. In the early stages of potassium deficiency, the fruit of both varieties had a normal external appearance but many ripe Klondike fruits had dead calyces. In more advanced stages of potassium deficiency, the wilting and drying up of the pedicels and peduncles resulted in considerable shriveling of fruits.

DAVIS and HILL (3) described potassium deficiency symptoms (variety not mentioned) in the following manner: "In early stages the plants were dark green in color which lasted well into the fruiting season; however, they were smaller and less vigorous than those receiving complete nutrients.

The leaves gradually lost their luster and by the end of the fruiting season began to curl. A bronzing and considerable purpling was observed on the under surface of the leaflets by late summer."

HOAGLAND and SNYDER (6) in studying potassium deficiency of the Marshall, Ohmer, and Klondike strawberries did not find marginal scorch prominent in any of these varieties, but reported bronzing and necrosis of the petiole and base of the blade.

CALCIUM DEFICIENCY.—In both the Blakemore and Klondike varieties, symptoms of calcium deficiency first appeared as injury to the roots followed by injury to the leaves. A marked varietal difference in the appearance of

FIG. 6. Symptoms of phosphate and calcium deficiencies in Blakemore strawberry plants.
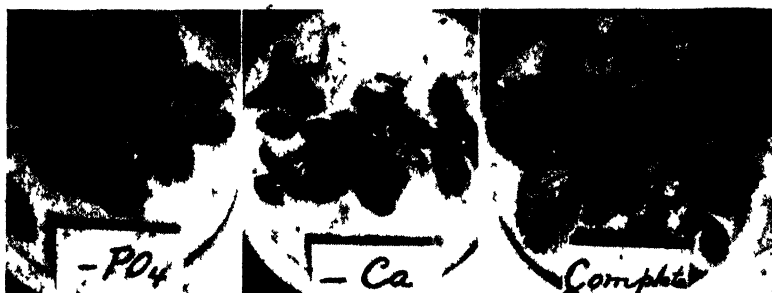
FIG. 7. Symptoms of phosphate and calcium deficiencies in Klondike strawberry plants.

calcium deficient leaves was evident. Calcium deficient Blakemore plants made much more growth than did the Klondike plants (figs. 6 and 7). Symptoms in the Blakemore foliage, however, were evident as in figure 4. The younger leaves were much deformed and crinkled, and exhibited tip burn. In contrast, the younger Klondike leaves were not crinkled but the older leaves lost their luster and became somewhat mottled. Later these older leaves became flaccid and internal breakdown of tissue was evident, suggesting that calcium had been translocated to some extent from them to the younger leaves. The fruits of calcium deficient plants of both varieties were very much deformed while still green and were still small when ripe.

The calyces of the ripe fruit of the Klondike variety all died, a condition also noted in potassium deficiency.

DAVIS et al. (4) reported that calcium deficient plants of the Parson Beauty strawberry grew vigorously during the summer and did not develop leaf blotch until early fall, after which leaf growth was restricted.

MAGNESIUM DEFICIENCY.—Early stages of magnesium deficiency in the Klondike foliage were characterized by the downward and inward rolling of the yellowish-green leaf margins. In later stages the upper surface of the leaf blades between the veins became a yellowish-orange color except in the region along the midrib. Brown necrotic areas appeared on the under surface of these leaves. These symptoms also appeared in magnesium deficient Blakemore foliage.

DAVIS et al. (4) reported that in summer the magnesium deficient series were the most vigorous of his experimental plants. The large leaves were of normal color until late August when characteristic brown patches, mostly confined to the leaf margins, developed. Abscission of these brown patches gave the leaves a ragged appearance. The petioles were always longer than those in the normal series.

PHOSPHATE DEFICIENCY.—Early stages of phosphate deficiency in both varieties were characterized by an intensified blue-green coloration of the foliage. This was accompanied by reddening of the leaf margins. In later stages the entire surface of the older leaf blades became bronzed and purpled due to the red pigments overlying the blue green. The petioles became red and brittle and the midribs and veins on the under surface of leaf blades showed the purple coloration that suggested a physiological nitrogen deficiency. The leaves were not as thick as the leaves of normal plants and the petioles were shorter. In more advanced stages of phosphate deficiency the plants were much dwarfed and the older leaves became brown and dry. DAVIS et al. (4) observed similar symptoms in the Parson Beauty variety in Canada.

NITROGEN DEFICIENCY.—In early stages of nitrogen deficiency the serrations at the margins of older leaves became red. As nitrogen deficiency progressed, the younger leaves developed more slowly and appeared yellowish green. Later a reddening gradually extended over the entire leaf surface. The petioles, which were shorter than in the plants receiving complete nutrients, became red and brittle. In advanced stages of nitrogen deficiency the older leaves became light yellow with browning and necrosis of localized areas. The ripe fruit was small and the plants developed few feeding roots. In general, these nitrogen deficiency symptoms are in agreement with those described by DAVIS and HILL (2) and by DAVIS et al. (4).

For the Aroma strawberry, LONG and MURNEEK (7) found that the nitrogen content of leaves decreased rapidly during senescence, with a simultaneous increase in the roots and stems. They further reported that the roots accumulated 30 to 40 per cent. of the total amount of nitrogen found in the plant during the winter.

DIFFERENCES IN SYMPTOMS

The mineral deficiency symptoms in the leaves of the Klondike and the Blakemore varieties as grown under the conditions reported herein are summarized in table III. Where there were differences in the symptoms between the young and old leaves such differences are listed. Varietal differences were greatest in the symptoms of calcium and potassium deficiencies. These differences were evident in the young leaves for calcium deficiency,

TABLE III

NUTRIENT DEFICIENCY SYMPTOMS IN LEAVES OF TWO VARIETIES OF STRAWBERRIES

NUTRIENT DEFICIENCY	VARIETY	SYMPTOMS
Calcium	Klondike	Young leaves: dwarfed, with marginal scorch, followed by death of buds. Mature leaves*: loss of luster and turgor, followed by mottling.
	Blakemore	Young leaves: dwarfed and crinkled near tips. Mature leaves: loss of luster and turgor followed by yellowing.
Potassium	Klondike	Mature leaves: progressive purpling of midrib and petiole, followed by necrosis of petiole.
	Blakemore	Mature leaves: margins of leaf blades scorched and rolled upward.
Magnesium	Klondike and Blakemore	Mature leaves: chlorosis of outer portion, followed by downward rolling of leaf margin.
Phosphate	Klondike and Blakemore	Young leaves: dark blue-green, and retarded in growth. Mature leaves: intensified blue-green accompanied by reddening of the leaf margins, the leaf blades later becoming bronzed and purpled and petioles bright red. Midribs and veins on under surface of leaf becoming purpled.
Nitrogen	Klondike and Blakemore	Young leaves: in more advanced stages, light green to yellow; growth restricted. Mature leaves: in early stages, the serrations of older leaves redden. Later the entire leaf surface reddens, the older leaves finally becoming bright yellow accompanied by necrosis and browning of localized areas.

* A mature leaf as used herein refers to fully expanded turgid leaves.

and in the mature leaves for potassium deficiency. No varietal difference in symptoms was observed for magnesium, phosphorus, or nitrogen deficiency. HOAGLAND and SNYDER (6) found Nick Ohmer strawberry to be less susceptible than Marshall to nutrient deficiencies, and Klondike to be the variety least susceptible to boron deficiency.

Discussion

The composition of the leaves of plants given a complete nutrient solution for about 2 months differed from that of dormant plants at the beginning of the experiment chiefly in the greater content of potassium. Analysis of

the leaves of plants in the deficiency tests after symptoms were evident indicates that their calcium, magnesium, and phosphorus content, as well as potassium content, may vary greatly depending on the supply. It would seem, therefore, that foliar analysis as an index to the nutrient condition of the plant might be practical.

In general, the nutrient content of the strawberry fruit followed that of the leaves but was not so high. Characteristic foliage symptoms of nutrient deficiencies were observed. For the most part they correspond to those observed in the strawberry by other investigators but additional symptoms were recorded. Particularly evident were the differences in the calcium and potassium deficiency symptoms for the Klondike variety as compared with the Blakemore. This indicates the need for a study of the symptoms for different varieties in order to make a correct diagnosis of deficiencies.

Summary

1. Foliar symptoms of potassium, calcium, magnesium, phosphate, and nitrogen deficiencies are described for both Blakemore and Klondike strawberry varieties during the fruiting stage of growth.

2. Marked varietal differences in potassium and calcium deficiency symptoms are noted and illustrated.

3. Soluble minerals were determined in the foliage and fruit of plants receiving the various nutrient treatments, and the results obtained are illustrated.

4. In the complete nutrient treatment of each variety the potassium concentration in relation to other soluble minerals in the fruit was high as compared with the same relationship in the leaves.

5. There were only traces of soluble phosphate in the foliage and fruit at the time phosphate deficiency symptoms were evident.

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LITERATURE CITED

1. BURKHART, LELAND, and PAGE, N. R. Mineral nutrient extraction and distribution in the peanut plant. Jour. Amer. Soc. Agron. **33**: 743–755. 1941.
2. DAVIS, M. B., and HILL, H. Nutritional studies with Fragaria. Sci. Agr. **8**: 681–691. 1928.
3. ————————, and ————————. Nitrogen, phosphoric acid, and potash starvation at different stages of growth of Fragaria. Canada Dept. Agr. Pamphlet no. 96. 1928.
4. ————————, ————————, and JOHNSON, F. B. A study of the effect of deficient and excess potassium, phosphorous, magnesium, calcium, and sulphur. Sci. Agr. **14**: 411–432. 1934.

5. GARDNER, V. R.   Studies in the nutrition of the strawberry.   Missouri Agr. Exp. Sta. Res. Bull. 57.   1923.

6. HOAGLAND, D. R., and SNYDER, W. C.   Nutrition of strawberry plant under controlled conditions.   Proc. Amer. Soc. Hort. Sci. **30**: 288–294.   1933.

7. LONG, J. H., and MURNEEK, A. E.   Nitrogen and carbohydrate content of the strawberry plant.   Missouri Agr. Exp. Sta. Res. Bull. 252.   1937.

8. WALLACE, T.   The nutrition and fertilizing of soft fruits.   Imp. Bur. Fruit Production Tech. Communication **6**: 52–87.   1936.

AN INSTALLATION OF LARGE SAND–CULTURE BEDS SURMOUNTED BY INDIVIDUAL AIR–CONDITIONED GREENHOUSES[1]

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JAMES O. IVIE, AND GEO. R. HILL

(WITH TEN FIGURES)

In this series of papers, various phases of the relations of sulphur to plant growth are being considered. Special consideration is given to the rôle of sulphur dioxide. This study was initiated in 1935–1936, when experiments were carried out at Logan, Utah (**8, 9**), in a non-industrial atmosphere sufficiently free from sulphur compounds to permit the study of the effects of low concentrations of sulphur dioxide on alfalfa. In order to continue the studies at this laboratory, it has been necessary to set up special equipment to control the sulphur supply, both to the roots and to the leaves of the vegetation. This has been accomplished by the construction of a group of large automatically-irrigated, sand-culture beds surmounted by individual greenhouses, supplied with washed and filtered air.

The utility of the sand bed is well established in solution-culture work, primarily because the sand provides support for the plants and permits good aeration of the roots, as well as control of soluble materials available to them. Further, it is not difficult to irrigate the plants uniformly. Normal growth of the vegetation and good yields have been repeatedly observed. For example, ARNON and HOAGLAND (**1**) grew tomatoes in the greenhouse in comparable sand, water, and soil cultures. They obtained excellent growth in all three media, but the yield of fruit was somewhat higher in the sand than in the water or soil. In our experience, root respiration measurements and transpiration measurements can be readily carried out in the sand bed. Also, it is not difficult to harvest the roots quantitatively.

In addition to the usual sand-cultures in pots, crocks, or on greenhouse benches, special small and large sand containers, and equipment for irrigating them automatically on the surface, have been described by EATON (**4, 5, 6**) and by CHAPMAN and LIEBIG (**3**). Subirrigated sand and gravel beds have been used by BIEKART and CONNORS (**2**) and by WITHROW and BIEBEL (**11, 12**). Some of these installations were designed for scientific studies in plant nutrition; others for commercial crop production.

The following features were sought for the equipment described in this paper:

1. Sand beds large enough to grow a number of plants to maturity, with root systems of as nearly normal size as possible.

2. Airtight greenhouses surmounting the beds, in which the gaseous environment could be controlled.

[1] This is the third of a series of papers on ''The Effect of Prolonged Low Concentrations of Sulphur Dioxide Upon Plants.''

3. Containers with insoluble walls that would supply as small an amount of nutrients as possible.

4. Automatic irrigation.

5. Ease of control of concentrations and volumes of the nutrient solutions, and of air with known sulphur dioxide concentrations.

6. Facilities for root respiration and photosynthesis measurements.

Installation

A diagram of one unit of the equipment is given in figure 1, and a section of the general assembly is shown in figure 2. Figure 3 is a photograph of
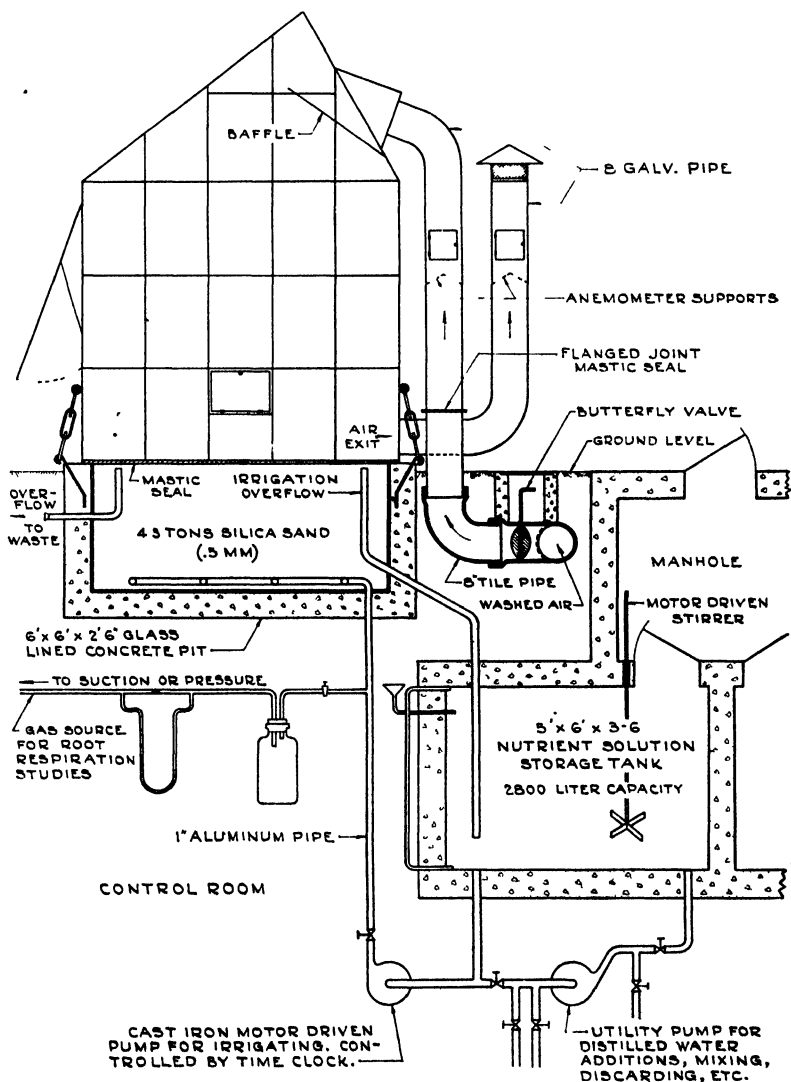
FIG. 1. Diagrammatic elevation of one unit of the sand culture equipment.

the installation, looking southwest, and showing, in addition to the green-houses, the location of the air-washing unit, the recorder room, and a large concrete pit for storing and treating the sand. Figure 4 shows the green-houses filled with first crop alfalfa on April 29, 1941.
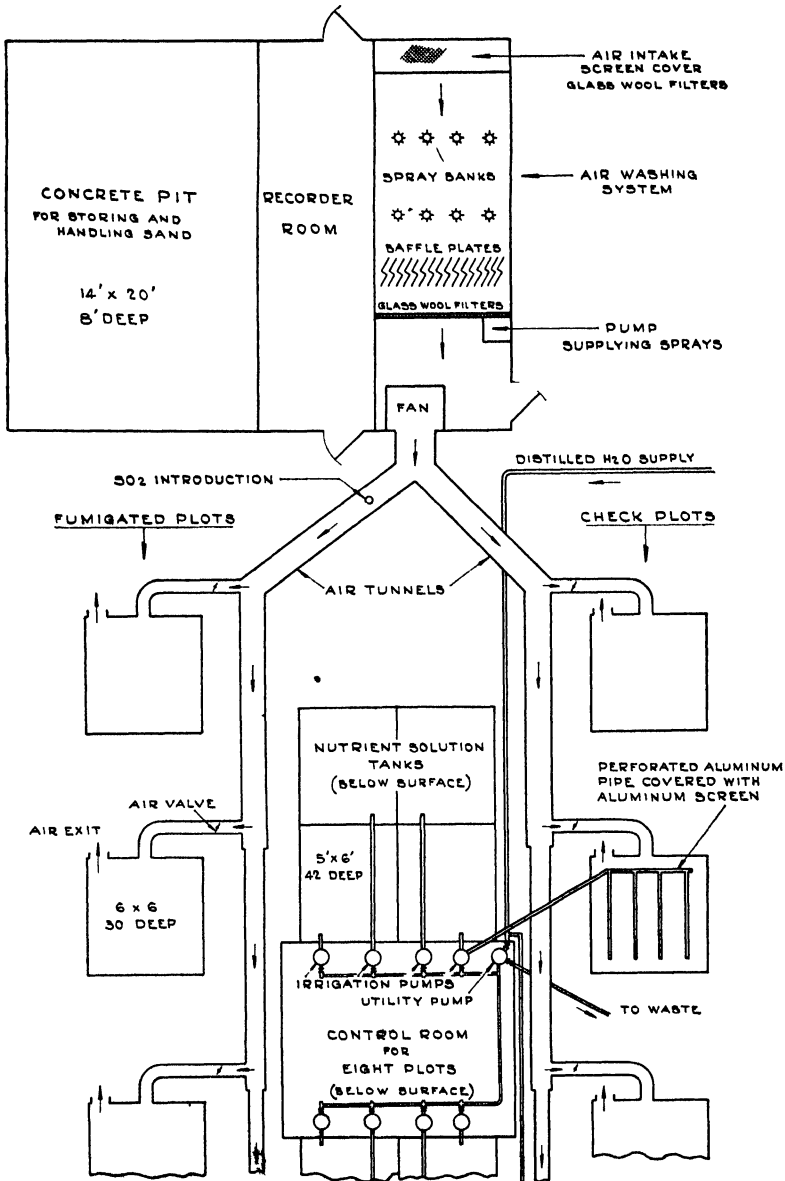
FIG. 2. Diagrammatic plan of the sand-culture and accessory equipment. There are sixteen sand beds and two control rooms.

The sand beds are concrete boxes 6′ × 6′ × 2.5′, set about 27 inches into the ground. The nutrient supply tanks are enclosed concrete boxes 5′ × 6′

× 3.5′, placed below the sand beds and connected with them by aluminum pipe through time-clock controlled cast iron motor pumps. The insides of all of these boxes were coated with a heavy layer of asphalt tar, but before the 1942 season started the walls of the sand beds were completely covered by plate glass mounted in tar. More recently the walls of the solution tanks were also covered by plate glass. The nutrient solution is introduced at the bottom of the bed through a manifold of aluminum pipe (fig. 10) covered with a fine aluminum screen to exclude the sand, and the overflow liquid is returned from the top of the sand to the supply tank. A second overflow is provided so that the water can be drained off to waste at the top of the bed when it is desired to wash the sand. A sample of the initial overflow liquid is collected automatically. The solution is allowed to overflow for 5 to 10

FIG. 3. General view of the installation looking southwest. In foreground is the air washing room, the recorder room, and the utility sand pit.

minutes before the pump is stopped and the excess water is drained back through the pump, requiring about forty minutes. A satisfactory displacement of the old solution and washing of the sand is thus obtained. One irrigation per day is usually sufficient.

The complete installation (fig. 2) consists of 16 sand beds and their individual supply tanks. Two underground laboratories provide access to the motor pumps (fig. 5) and the accessory equipment, which includes facilities for sampling the solutions, adding concentrated nutrients, handling and mixing the solutions, measuring and making up evaporation losses. Air pumps are also provided to force air either up or down through the sand in connection with sulphur dioxide fumigations and root respiration measurements.

On the smooth top edge of each sand box is placed a gastight greenhouse (fig. 6), made of commercial steel window frames, mounted on an angle iron framework. The joint with the concrete surface is made with mastic. This greenhouse has an 8″ intake pipe leading up the north side and entering near the top. A similar outlet pipe emerges from the bottom. For ready access to the inside, the greenhouse is provided with four 10″ × 14″ doors, one on each side, and also one gastight door large enough to admit a man. The whole structure can be removed from the sand bed with a crane (fig. 7).

The ventilation system includes air washing and filtering equipment (fig. 3). The air is washed with tap water in a concrete chamber 7′ × 7′

Fig. 4. View of the plant chambers containing alfalfa on April 29, 1941.

× 14′, using a double bank of 100 sprays, operated by a 3 H.P. motor pump. The spray is removed by the usual galvanized iron eliminator plates, and the air is filtered through glass wool filters before and after entering the spray chamber. A 9,000 c.f.m. blower then delivers the air to the 16 greenhouses through underground tile pipes. Dampers and air valves are provided to control the air flow so that it is equally distributed to the different greenhouses. No attempt has been made to thermostat the plant chambers, but their temperature usually is the same as, or a little below, outside air temperature. Continuous analysis of the washed air has failed to show a detectable amount of sulphur dioxide. The limit of this analytical method is about 2 parts per billion.

Ottawa (Illinois) silica sand is used in the beds. This material consists of clear well-rounded grains, 0.5–0.6 mm. in diameter, assaying 99.8 per cent. silica. The remaining 0.2 per cent. is nearly all iron and aluminum

oxides. It has proven to be entirely satisfactory as to particle size, and pure enough for most purposes.

Each bed contains 4,000 kg. of sand. In an experiment to determine the amount of moisture held by the sand, a bed was flooded and allowed to drain for 2 hours. On sampling with a tube, 11.4 per cent. of moisture was found. After 24 hours, the sand retained 9.4 per cent. water. When air was drawn down through the bed over night after irrigating, 6.9 per cent.

FIG. 5. Solution pumps showing interconnections and discharge pipes to the sand beds.

water was found next day, and 4.8 per cent. after about 1 week. The permanent wilting percentage of this sand has not been determined, but it is probably less than 2 per cent. The bed will, therefore, retain at least 100 to 350 liters of readily available moisture, under different conditions. The maximum amount of evaporation that has been observed with a large crop of alfalfa was about 30 liters per day. This would represent less than 10 per cent. of the water available if the sand were irrigated daily.

At harvest, the root systems can be removed nearly intact if desired. Figure 9 is a photograph of an alfalfa root partially washed free of sand. Figure 10 shows the intermeshed roots of three winter wheat plants and one barley plant, which have been washed free of sand in place. Note part of the irrigation manifold in the bottom of the pit. The tarred wall of the pit in figure 9 may be compared with the plate glass wall in figure 10. If the roots are not desired intact, it is simpler to pull out the larger portions and

FIG. 6. Gieenhouse unit, showing access to inside and the strips of sheet aluminum covering sand.

separate the hair roots from the sand by means of a large screen, agitated in the surface of a pool of water (fig. 8). After further washing, the sand can be used again. One prolonged washing with 0.1 N nitric acid has usually been employed. It has been possible to secure a satisfactory control of green algae by the use of strips of sheet aluminum between the plants.

Nutrient solutions

The principal constituents of the nutrient solutions have had approximately the proportions but not more than half the concentrations suggested by HOAGLAND (7). It has been necessary also to make modifications

FIG 7. Greenhouse unit surmounted by the crane for removing the house from the sand pit.

to secure the desired range of sulphur and hydrogen ion concentrations. In general the basic solution has been as follows:

$Ca(NO_3)_2$ 0.002 M
KNO_3 0.002 M
$Mg(NO_3)_2$ 0 0008 M

To this solution, phosphoric acid or potassium phosphate is added to give a phosphate concentration of 0 0001 M or about 3 p.p m. More recently the
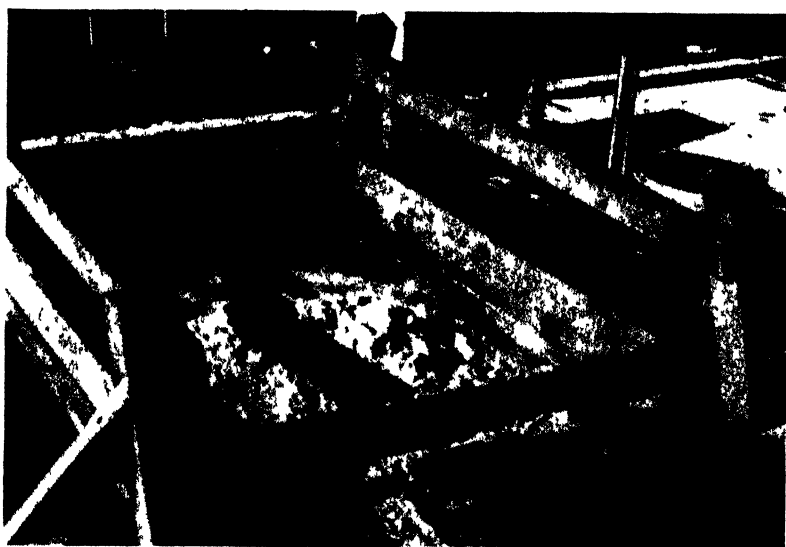
FIG. 8.   Equipment for screening the sand to separate the hair roots from the sand.

FIG. 9.   Alfalfa root partially washed free of sand (1941).
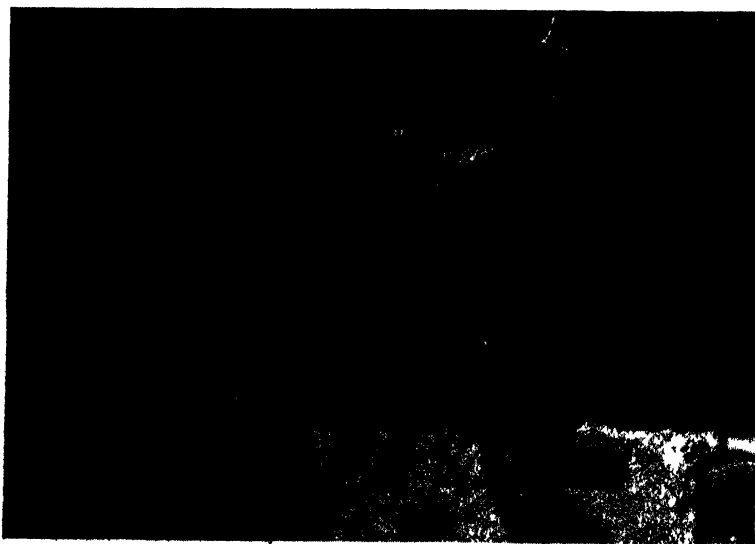
FIG. 10.   Roots of four grain plants washed free of sand in place (1942).   Note part of irrigation manifold in bottom of the pit.

concentration of the potassium nitrate has been increased to 0.004 M and that of the calcium nitrate reduced to 0.0012 M in accordance with the suggestion of ARNON and HOAGLAND (1) that the ions are absorbed by the plants in about these proportions.

Ammonia or potassium hydroxide is then used to adjust the pH, which is subsequently maintained by the proper nitrate-ammonia ratio as suggested by TRELEASE and TRELEASE (10). It has not been practicable to maintain a phosphorous concentration higher than 3 p.p.m. on account of precipitation. Metaphosphate was tried and seemed promising for a time, but it soon reverted to orthophosphate and precipitated. The desired amount of sulphate is added as potassium, magnesium, or calcium salt. Iron is maintained between 0.15 and 0.5 p.p.m. by repeated additions of ferric nitrate, and the usual "trace" elements are also added. Lower nutrient levels have been secured by dilution of the basic solution without a corresponding dilution of the sulphur, phosphorus, iron, and trace elements. It is necessary to make up these solutions with distilled water, since the best natural water contains too much sulphur for these studies. A 20-gallon-per-hour still and a 3,000-gallon storage tank, provide the large amount of water needed.

The installation has been in use since 1939. Subsequent papers in this series will describe the experiments conducted in it.

Summary

A large sand-culture installation is described, with automatic equipment for subirrigating the beds and facilities for measuring transpiration, photosynthesis, and root respiration. The beds are surmounted by airtight individual greenhouses, supplied with washed and filtered air free from sulphur dioxide, thus permitting control of the gaseous environment.

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LITERATURE CITED

1. ARNON, D. I., and HOAGLAND, D. R. Crop production in artificial culture solutions and in soils with special reference to factors influencing yields and absorption of inorganic nutrients. Soil Sci. **50**: 463–485. 1940.

2. BIEKART, H. M., and CONNORS, C. H. The greenhouse culture of carnations in sand. New Jersey Agr. Exp. Sta. Bull. 588. 1935.

3. CHAPMAN, H. D., and LIEBIG, G. F. Adaptation and use of automatically operated sand culture equipment. Jour. Agr. Res. **56**: 73–80. 1938.

4. EATON, FRANK M. A large sand culture apparatus. Soil Sci. **31**: 235–241. 1931.

5. ——————. Automatically operated sand-culture equipment. Jour. Agr. Res. **53**: 433–444. 1936.

6. ——————————. Plant culture equipment. Plant Physiol. **16**: 385–392. 1941.

7. HOAGLAND, D. R.   Optimum nutrient solutions for plants.   Science n.s. **52**: 562–564.   1920.

8. THOMAS, MOYER D., and HILL, GEO. R.   The continuous measurement of photosynthesis, respiration, and transpiration of alfalfa and wheat growing under field conditions.   Plant Physiol. **12**: 285–307. 1937.

9. ——————————, and ——————————.   Relation of sulphur dioxide in the atmosphere to photosynthesis and respiration of alfalfa.   Plant Physiol. **12**: 309–383.   1937. ·

10. TRELEASE, SAM F., and TRELEASE, HELEN M.   Physiologically balanced culture solutions with stable hydrogen-ion concentration.   Science n.s. **78**: 438–439.   1933.

11. WITHROW, R. B., and BIEBEL, J. P.   A subirrigation method of supplying nutrient solutions to plants growing under commercial and experimental conditions.   Jour. Agr. Res. **53**: 693–701.   1936.

12. ——————————, and ——————————.   Nutrient solution methods of greenhouse crop production.   Purdue University Agr. Exp. Sta. Circular 232.   1937.

THE UTILIZATION OF SULPHATE AND SULPHUR DIOXIDE FOR THE SULPHUR NUTRITION OF ALFALFA[1]

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T. R. COLLIER, AND GEO. R. HILL

(WITH FIVE FIGURES)

Introduction

Though sulphur has long been recognized as an essential element in plants, particularly as a constituent of the proteins, it has not received much consideration in fertilizer studies. As shown by the work of HART and PETERSON (3), this was partly due to the older practice of determining sulphur in the ash of vegetation, which gave low results and did not indicate the true sulphur needs of the plants. In practical agriculture, sulphur deficiency has probably been uncommon, at least during the last century, because of important additions of the element from the atmosphere, and important, though unintentional, additions in commercial fertilizers.

Regions of sulphur deficiency, however, have been shown to exist in the Pacific Northwest (6), in Western Canada, and in Northern Minnesota (2). The location of these regions where winds from industrial areas seldom blow, seems to emphasize the importance of atmospheric sulphur in supplying the needs of those regions in which there is industrial activity and favorable prevailing winds. ALWAY (1) has given an historical review of sulphur in agriculture in his presidential address to the American Society of Agronomy, in which this viewpoint was advanced and in which it was suggested that part of the sulphur requirements of plants might be supplied by direct absorption of sulphur dioxide into the leaves, as well as indirect absorption after the gas has been taken into the soil and changed to sulphate. SETTERSTROM, ZIMMERMAN, and CROCKER (8) have submitted evidence that sulphur dioxide can increase the growth rate of sulphur-deficient plants.

In this paper a study has been made, using the large sand-culture installation (9), of the sulphur needs of alfalfa, and the possibility has been explored of substituting sulphur dioxide for sulphate as the source. The experiments were planned to determine the effect of sublethal concentrations of sulphur dioxide on the growth of alfalfa under a wide range of conditions—such as different hydrogen ion concentrations and different concentrations of the principal nutrient elements. In particular, the effects of sulphur dioxide on sulphur-deficient vegetation were sought.

Methods

1939 EXPERIMENTS

Twelve plots of alfalfa were studied in 1939. Seedling plants were grown in the greenhouse in complete nutrient solutions in silica sand in

[1] This is the fourth of a series of papers on ''The Effect of Prolonged Low Concentrations of Sulphur Dioxide upon Plants.''

deep narrow wooden boxes 4″ × 30″ by 18″ deep. Seed of Turkestan alfalfa, derived from a single plant selection, and furnished by the Utah Experiment Station, was planted in February. The plants were topped in April and prepared for transplanting by removing one side and the bottom of each box and washing away the sand with a spray of water. The seedlings were sorted and those of uniform size were distributed on trays so that all the plots received 25 plants which were as nearly the same size as possible. The average fresh weight of the seedlings was about 5 grams per plant; dry weight about 0.7 grams per plant. The transplanting was completed by inserting an 18″ metal planting tool enclosing the seedlings into the moist sand. The tool was then separated into two parts and removed, leaving the seedling in place. These transplanting operations were carried out with negligible injury to the plants.

All the plots were supplied with a base nutrient solution of the approximate composition already indicated (9). Six of the plots were maintained at a pH of about 5.5 and the other six at about pH 7.0. Four plots had no sulphur added intentionally to the nutrient solution; four had sulphate equivalent to 5 p.p.m. of sulphur; and four 90 p.p.m. of sulphur. Thus there were six pairs of plots with low and high pH and with low, medium, and high sulphate in the nutrient solution. One plot of each pair was fumigated with sulphur dioxide at a concentration of about 0.1 p.p.m. for 6 to 7 hours each day (usually from 9 A.M. to 4 P.M.) and six days each week throughout the growth period. The other plot of each pair was unfumigated. Four crops were grown during the season, and after cutting the fourth crop, the roots were also harvested.

1940–1941 EXPERIMENTS

Seedling alfalfa plants, grown as in 1939 were transplanted on April 25, 1940 to 12 plots of new sand and 4 plots of used sand that had been washed and steam sterilized. The seedlings had been treated with Rhizobium, supplied by the University of Wisconsin, and were nodulated when transplanted.

An attempt was made after transplanting the seedlings to grow one-half the plots in a solution 40 per cent. of the strength of the 1939 solution, and the other half in a solution of 20 per cent. strength. The plants did not grow as well as in 1939 and it was decided on May 8 to raise the concentration of the former group 2.5 fold. The weaker solutions were not modified until June 3, when one millimol of calcium nitrate was added to them, because the plants appeared to be very unthrifty. This group of plots was placed on the 1939 level of nutrient concentration for the second crop and maintained at that level throughout the remainder of the experiment. The other plots were also maintained at this level for the second crop in 1940; thereafter the concentration of the solution was progressively lowered until it finally reached 3 per cent. of this value. The pH of all the solutions was maintained at about 5.5 during the two years. Four levels of sulphate concentration were established in the nutrient solutions. Four plots had

no sulphate added intentionally. The other three groups had nominal sulphate concentrations equivalent to 0.8 p.p.m., 1.5 p.p.m., and 10 p.p.m. sulphur. There were, therefore, 8 pairs of plots with low and high nutrient levels and low, medium low, medium, and high sulphate sulphur in the nutrient solution. It should be noted that these designations of the sulphate concentrations do not have the same values as in 1939. One plot of each pair was fumigated with sulphur dioxide as in 1939, the other plot being untreated. Four crops were harvested in 1940; then five crops and finally the roots in 1941.

Results

Cultural data

Average concentrations of the principal nutrients, determined by analysis of the solutions, for each crop in 1939 are summarized in table I and the corresponding data for 1940–1941 are given in table II. These tables include the fumigation data. In the 1940–1941 data, the "low nutrient level" group of plots for the first crop in 1940, became the "high nutrient level" group for the second and subsequent crops and vice versa. It is particularly interesting to note the low nutrient concentrations employed in 1941, on which good growth was obtained, due probably to the well-established root systems. Even the highest of these concentrations was inadequate for the first crop in 1940 when the root systems were small.

Growth of the alfalfa was vigorous. Four crops were harvested during each first year and five crops during the second year. The plant chambers were repeatedly filled with vegetation, as already illustrated (9). Maximum yields were at the rate of 10 tons per acre of dry alfalfa in 1939, 6.5 tons in 1940, and 15 tons in 1941. The sulphur dioxide fumigations did not cause any acute markings, but chlorotic markings usually developed on the older leaves to the extent of about 5 to 10 per cent. of the leaf area of the plot as described later.

Table III gives the chlorophyll content of all the crops, determined on fresh leaves by the method of SCHERTZ (7). These leaf samples were obtained by separating the leaves and stems of a large representative portion of the plots at harvest, as already described (11). The table confirms the visual observations, that the leaves of the sulphur-deficient plants were not as dark green in color as the plants supplied with adequate sulphur, either from the nutrient solution or from sulphur dioxide. The "low sulphur-high nutrient" check plot particularly, had at all times a distinctly yellow cast. An exception was the "high sulphur-low nutrient" fumigated plot, which became chlorotic on the last three crops in 1941, due possibly to the development of root disease.

Considerable difficulty was experienced after the first crop in 1939 due to aphis, which were sufficiently numerous in a few cases to reduce somewhat the yield of the crops. Thrips also were present. In 1940–1941 aphis were completely eliminated by the nicotine sprays used to control the more

TABLE I

CULTURAL DATA FOR 1939 EXPERIMENTS

Crop	Dates Harvested	pH		Sulphate Concentration			SO₂ Fumigation		Average Nutrient Concentration						NH₃	
		High	Low	High	Medium	Low	Total Time	Concentration	K	Ca	Mg	P	Fe	NO₃	High pH	Low pH
					p.p.m. S		hours	p.p.m.	m. mol.	m. mol.	m. mol.	m. mol.	m. mol.	m. mol.	m. mol.	m. mol.
1st	June 21–22	6.73	5.52	83.0	3.9	0.85	293	0.11	3.5	3.2	1.15	0.25	0.008	8.9	0.04	0.56
2nd	July 26–27	7.06	5.58	88.0	4.2	0.61	179	0.10	3.4	2.8	1.18	0.18	0.007	8.2	0.00	0.34
3rd	Sept. 6– 7	7.05	5.69	88.0	4.4	0.46	225	0.10	2.9	2.4	0.94	0.08	0.005	8.1	0.00	0.22
4th	Oct. 26–27	7.04	5.65	80.0	3.6	0.26	223	0.09	2.5	2.5	1.06	0.04	0.003	6.8	0.14	0.74

TABLE II
CULTURAL DATA FOR 1940 AND 1941 EXPERIMENTS

Crop	Date Harvested	Sulphate Concentration (p.p.m. S)				SO₂ Fumigation		Average Nutrient Concentration (m.mol.)						
		High	Medium	Medium-Low	Low	Total Time (hours)	Concentration (p.p.m.)	K	Ca	Mg	P	Fe	NO₃	NH₃
High nutrient level														
1940														
1st	July 1	10.9	2.24	0.93	0.75	240.0	0.09	2.69	2.42	1.16	0.06	0.005	8.00	1.19
2nd	Aug. 9	10.9	2.13	0.90	0.53	182.0	0.11	2.24	1.76	0.70	0.04	0.007	7.30	0.82
3rd	Sept. 17	8.8	1.28	0.61	0.48	167.0	0.12	2.33	1.78	1.09	0.05	0.008	7.30	0.17
4th	Nov. 13	8.4	1.21	0.61	0.36	347.0	0.12	1.77	1.52	0.85	0.03	0.003	5.40	0.07
1941														
1st	May 7	8.8	1.36	0.53	0.14	318.0	0.13	1.21	1.58	0.73	0.02	0.006	6.15	0.53
2nd	June 12	9.8	1.45	0.57	0.18	105.0	0.12	1.70	1.84	0.99	0.02	0.003	7.30	0.64
3rd	July 22	9.1	1.40	0.53	0.21	137.0	0.09	1.56	1.91	0.99	0.01	0.005	6.85	0.26
4th	Sept. 2	8.9	1.38	0.53	0.25	150.0	0.08	2.26	1.83	0.85	0.02	0.003	7.30	0.52
5th	Oct. 16	8.6	1.26	0.45	0.26	115.0	0.05	2.19	1.73	0.77	0.01	0.005	6.43	0.07
Low nutrient level														
1940														
1st	June 28	11.1	2.42	0.84	0.46	240.0	0.09	1.49	0.41	0.29	0.05	0.005	1.00	0.15
2nd	Aug. 8	9.0	1.16	0.61	0.35	182.0	0.11	2.88	1.84	1.00	0.06	0.008	8.30	1.04
3rd	Sept. 16	9.0	1.19	0.39	0.39	167.0	0.12	0.90	0.67	0.41	0.05	0.008	2.72	0.15
4th	Nov. 12	7.5	1.34	0.60	0.33	347.0	0.12	0.24	0.27	0.26	0.04	0.006	1.03	0.08
1941														
1st	May 6	9.1	1.46	0.49	0.19	318.0	0.13	0.08	0.19	0.04	0.02	0.006	0.56	0.12
2nd	June 11	10.3	1.66	0.59	0.18	105.0	0.12	0.21	0.22	0.11	0.03	0.004	0.79	0.11
3rd	July 21	9.1	1.35	0.56	0.22	137.0	0.09	0.11	0.21	0.08	0.03	0.006	0.60	0.04
4th	Sept. 1	8.9	1.65	0.66	0.28	150.0	0.08	0.09	0.12	0.06	0.02	0.004	0.26	0.05
5th	Oct. 15	9.0	1.78	0.56	0.31	115.0	0.05	0.09	0.10	0.03	0.01	0.006	0.19	0.01

TABLE III

CHLOROPHYLL CONTENT OF THE ALFALFA LEAVES

CROP	HIGH S CHECK %	HIGH S FUMIGATED %	MEDIUM S CHECK %	MEDIUM S FUMIGATED %	MEDIUM-LOW S CHECK %	MEDIUM-LOW S FUMIGATED %	LOW S CHECK %	LOW S FUMIGATED %	HIGH S CHECK %	HIGH S FUMIGATED %	MEDIUM S CHECK %	MEDIUM S FUMIGATED %	MEDIUM-LOW S CHECK %	MEDIUM-LOW S FUMIGATED %	LOW S CHECK %	LOW S FUMIGATED %
1939 — High pH (left) / Low pH (right)																
1st	1.92	1.97	1.60	1.90	......	......	1.50	1.70	2.03	1.93	1.90	1.98	......	......	1.64	1.74
2nd	2.21	2.23	2.10	2.14	......	......	1.91	2.10	2.30	2.27	2.30	2.10	......	......	1.90	2.25
3rd	2.20	2.20	2.12	2.05	......	......	1.82	2.02	2.31	2.15	2.37	2.24	......	......	1.90	2.25
4th	2.12	1.76	2.03	1.82*	......	......	1.32	1.79	2.04	2.07	2.12	1.91*	......	......	1.32	1.83
Average	2.11	2.04	1.96	1.98	......	......	1.64	1.90	2.17	2.11	2.17	2.06	......	......	1.69	2.02
1940 — High nutrient (left) / Low nutrient (right)																
1st	1.31	1.31	1.58	1.47	1.41	1.52	1.26	1.57	1.22	1.31	1.37	1.52	1.41	1.47	1.31	1.30
2nd	2.04	1.90	1.90	2.10	1.60	1.82	1.39	1.58	1.85	1.92	2.00	1.92	1.52	1.71	1.47	1.80
3rd	2.15	2.44	2.28	2.13	2.17	1.93	1.90	2.02	2.40	2.19	2.12	2.03	2.11	2.09	1.93	2.05
4th	1.50	1.55	1.69	1.50	1.55	1.41	1.39	1.40	1.51	1.44	1.35	1.35	1.67	1.46	1.52	1.56
Average	1.75	1.80	1.86	1.80	1.68	1.68	1.48	1.64	1.74	1.71	1.71	1.71	1.68	1.67	1.56	1.68
1941 — High nutrient (left) / Low nutrient (right)																
1st	1.52	1.95	2.01	2.00	1.96	1.92	1.49	1.79	1.84	1.77	1.67	1.67	1.61	1.50	1.49	1.45
2nd	1.71	1.65	2.13	1.91	1.81	1.58	1.01	1.60	1.85	1.67	1.77	1.67	1.77	1.45	1.39	1.69
3rd	1.45	1.37	1.46	1.58	1.37	1.35	0.90	1.20	1.34	1.20	1.37	1.35	1.10	1.06	1.07	1.09
4th	2.10	2.03	2.10	2.06	1.99	1.76	1.29	1.84	1.77	1.18	1.65	1.47	1.29	1.46	1.55	1.40
5th	1.83	1.82	1.84	1.83	1.84	1.75	1.05	1.36	1.43	1.17	1.74	1.64	1.55	1.44	1.44	1.06
Average	1.72	1.76	1.91	1.88	1.79	1.67	1.15	1.56	1.64	1.40	1.64	1.56	1.46	1.38	1.39	1.34

* Heavy infestation of aphis.

troublesome thrips. It is probable that thrips were responsible for some of the variability in the data to follow. Infestation was heavy and general and it was necessary to reduce their population at the beginning of each crop by nicotine sprays, because they attacked the growing points and interfered seriously with the development of the new shoots. It was im-
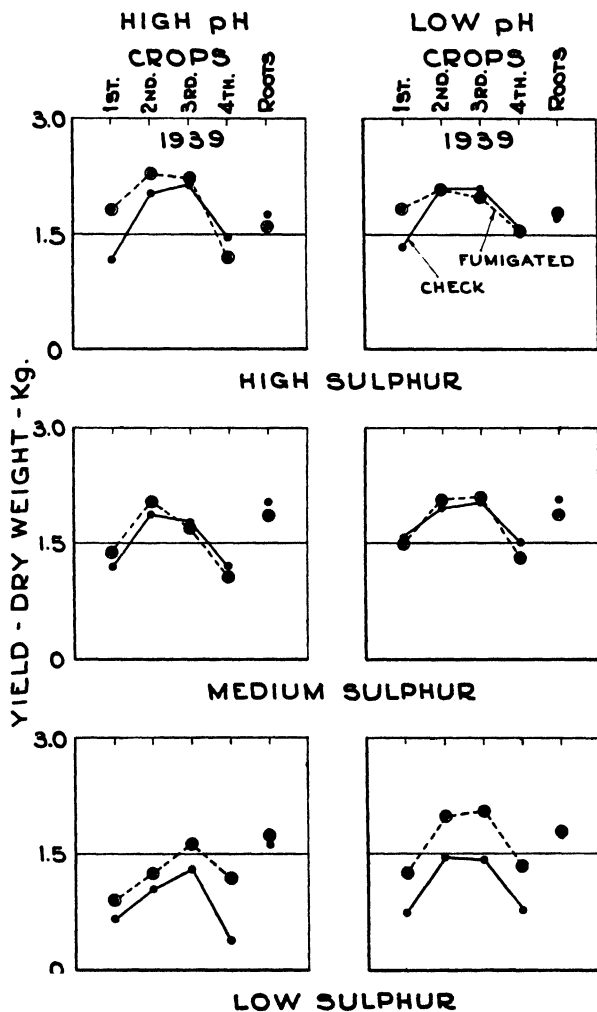
FIG. 1. Alfalfa crop yield data for 1939.

practicable to spray the larger plants, but the thrips seemed to be much less harmful at this stage. The nicotine sprays produced some typical foliar markings, which destroyed about 1 per cent. of the leaf area of all the plots.

YIELD

Yield data are presented as kilograms dry weight per plot for the 1939 crops in table IV and figure 1 and for the 1940–1941 crops in table V and figure 2. The yearly yields are summarized in table VI and figure 3. The

TABLE IV

Yield Data for 1939 Experiments (dry weight per plot)

CROP	High pH (7.0)						Low pH (5.5)					
	HIGH SULPHUR		MEDIUM SULPHUR		LOW SULPHUR		HIGH SULPHUR		MEDIUM SULPHUR		LOW SULPHUR	
	CHECK	FUMIGATED	CHECK	FUMIGATED	CHECK	FUMIGATED	CHECK	FUMIGATED	CHECK	FUMIGATED	CHECK	FUMIGATED
	kg.	kg.	kg.	kg.	kg.	kg.	kg.	kg.	kg.	kg.	kg.	kg.
1st	1.17	1.82	1.20	1.35	0.66	0.91	1.35	1.86	1.56	1.50	0.72	1.25
2nd	2.05	2.31	1.88	2.03	1.05	1.25	2.11	2.11	1.96	2.03	1.48	1.98
3rd	2.16	2.22	1.78	1.72	1.30	1.65	2.10	1.98	2.07	2.08	1.43	2.07
4th	1.44	1.19	1.20	1.07*	0.39	1.21	1.56	1.53	1.51	1.32*	0.77	1.35
Total crops	6.82	7.54	6.06	6.17	3.40	5.02	7.12	7.48	7.10	6.93	4.40	6.65
Roots	1.19	1.07	1.45	1.27	1.20	1.20	1.10	1.17	1.37	1.08	1.22	1.20
Crowns	0.58	0.55	0.62	0.59	0.43	0.55	0.61	0.58	0.70	0.59	0.52	0.58
Roots and crowns	1.77	1.62	2.07	1.86	1.63	1.75	1.71	1.75	2.07	1.67	1.74	1.78
Total	8.59	9.16	8.13	8.03	5.03	6.77	8.83	9.23	9.17	8.60	6.14	8.43

* Heavy infestation of aphis.

TABLE V

YIELD DATA FOR 1940–1941 (DRY WEIGHT PER PLOT)

| | HIGH NUTRIENT LEVEL | | | | | | | | LOW NUTRIENT LEVEL | | | | | | | |
| | HIGH S | | MEDIUM S | | MEDIUM-LOW S | | LOW S | | HIGH S | | MEDIUM S | | MEDIUM-LOW S | | LOW S | |
CROP	CHECK	FUMI-GATED	CHECK	FUMI-GATED	CHECK	FUMI-GATED	CHECK	FUMI-GATED	CHECK	FUMI-GATED	CHECK	FUMI-GATED	CHECK	FUMI-GATED	CHECK	FUMI-GATED
	kg.	kg.	kg.	kg.	kg.	kg.	kg.	kg.	kg.	kg.	kg.	kg.	kg.	kg.	kg.	kg.
								1940								
1st*	0.52	0.60	0.62	0.50	0.37	0.50	0.41	0.63	0.16	0.12	0.07	0.14	0.08	0.08	0.04	0.09
2nd	1.11	0.97	0.86	1.12	0.53	0.76	0.34	0.40	1.11	1.04	1.29	1.08	0.54	1.19	0.47	0.86
3rd	1.61	1.59	1.50	1.45	1.05	1.28	0.66	1.12	1.41	1.31	1.43	1.29	1.04	1.46	0.69	1.25
4th	1.63	1.49	1.44	1.20	1.06	1.21	0.58	1.14	0.94	0.86	0.95	0.84	0.85	0.95	0.55	1.02
Total	4.87	4.65	4.42	4.27	3.01	3.75	1.99	3.29	3.62	3.33	3.74	3.35	2.51	3.68	1.75	3.22
								1941								
1st	3.07	2.97	3.22	2.97	2.99	3.19	2.59	2.82	2.51	2.03	2.34	2.28	2.29	2.17	2.20	2.42
2nd	1.98	1.74	1.97	1.96	1.80	1.78	1.20	1.45	1.60	1.41	1.59	1.44	1.30	1.40	1.12	1.31
3rd	2.54	2.30	2.57	2.63	2.06	2.27	1.39	1.82	1.93	1.74	2.04	1.94	1.55	1.60	1.27	1.59
4th	2.42	2.07	2.08	2.12	1.90	2.00	1.31	1.70	1.24	0.98†	1.22	1.17	0.89	1.05	1.26‡	0.96
5th	1.31	1.23	1.14	1.21	1.16	1.13	0.74	0.81	0.73	0.51†	0.87	0.84	0.51	0.56	0.73‡	0.50
Total	11.32	10.31	10.98	10.89	9.91	10.37	7.23	8.60	8.01	6.67	8.06	7.67	6.54	6.78	6.58	6.78
Total crops	16.19	14.96	15.40	15.16	12.92	14.12	9.22	11.89	11.63	10.00	11.80	11.02	9.05	10.46	8.33	10.00
Roots	2.08	2.33	2.42	2.33	2.38	1.95	2.23	2.20	2.47	2.15†	2.47	2.29	2.59	2.38	2.65	2.33
Crowns	0.80	0.74	0.76	0.68	0.80	0.75	0.63	0.52	0.65	0.59	0.72	0.59	0.66	0.58	0.60	0.57
Roots and crowns	2.88	3.07	3.18	3.01	3.18	2.70	2.86	2.72	3.12	2.74	3.19	2.88	3.25	2.96	3.25	2.90
Total crops and roots	19.07	18.03	18.58	18.17	16.10	16.82	12.08	14.61	14.75	12.74†	14.99	13.90	12.30	13.42	11.58	12.90

* The yield data for low nutrient level, first crop 1940, were obtained from the plots from which all subsequent high nutrient level yield data were obtained, and vice versa.

† 4th and 5th crops were seriously affected by root disease.

‡ 4th and 5th crops had a nearly adequate supply of sulphur from an unknown source.

1939 data indicate a somewhat better growth of alfalfa at pH 5.5 than at pH 7, at the "low" and "medium" sulphur levels. At the "high sulphur level" the pH effect seemed to disappear. The reduced yield of the check
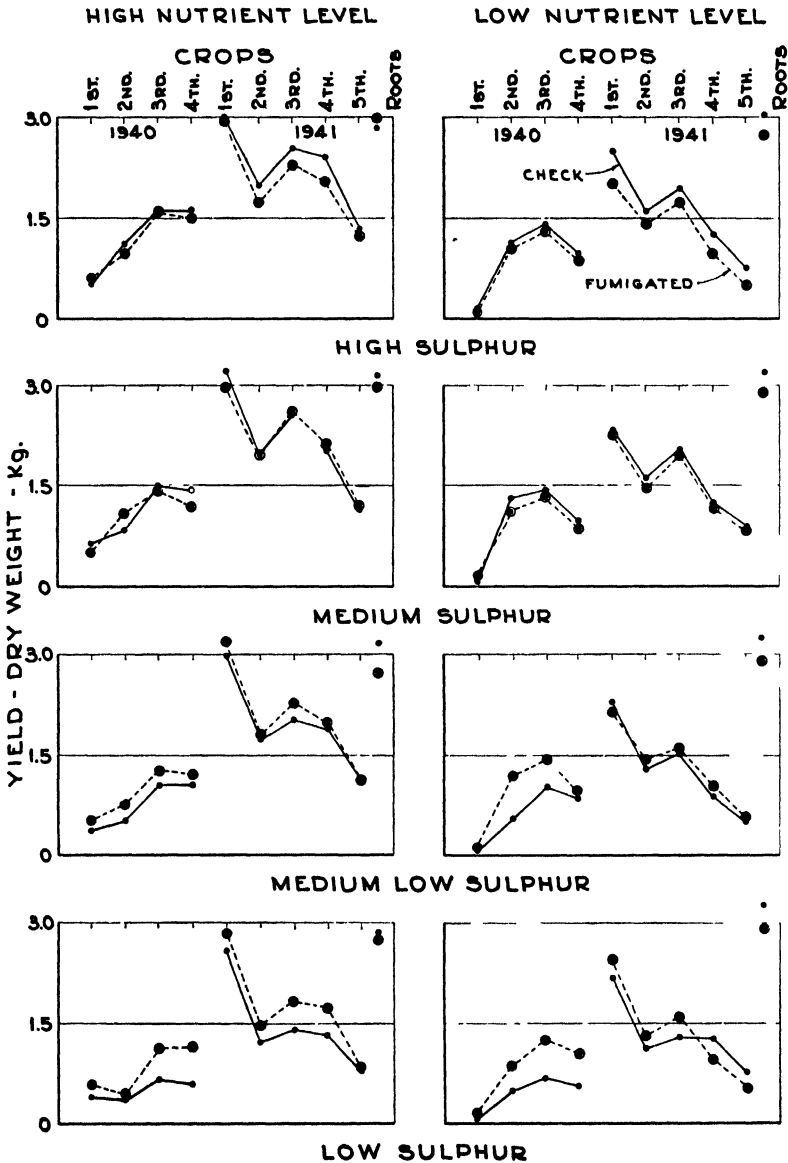
FIG. 2. Alfalfa crop yield data for 1940 and 1941.

plots at the low sulphur level was a definite indication of a deficient supply of this element. In most cases growth with "medium sulphur" was about the same as with high sulphur. The sulphur dioxide fumigations increased the yield markedly on all the low sulphur plots, but had little effect on the others, except for the anomalous increase of the first crop with high sulphur

for which no explanation is apparent. Two of the medium sulphur fumigated crops gave lower yields than the checks due to heavy infestations of aphis.

The 1940–1941 yield data (table V, fig. 2) indicate a sulphur deficiency in the low sulphur and medium-low sulphur groups of plots. In nearly all of these crops, the check yields were lower than the corresponding yields with medium sulphur and high sulphur and further, the fumigated plots almost invariably gave higher yields than the corresponding checks. The exception was the low nutrient, low sulphur check plot which on the fourth and fifth crops in 1941 not only gave larger yields than its fumigated mate,

TABLE VI

RATIOS OF THE AVERAGE YEARLY YIELDS OF THE FUMIGATED PLOTS TO THE CORRESPONDING CHECK PLOTS

	HIGH S	MEDIUM S	MEDIUM LOW S	LOW S	HIGH S	MEDIUM S	MEDIUM-LOW S	LOW S
				1939 data				
		High pH				Low pH		
Crops	1.11	1.02		1.48	1.05	0.98		1.51
Roots	0.90	0.88		1.00	1.06	0.79		0.98
Roots + crowns	0.92	0.90		1.07	1.03	0.81		1.02
Total	1.07	0.99		1.35	1.05	0.94		1.37
				1940 data				
		High nutrient				Low nutrient		
Crops	0.96	0.97	1.25	1.65	0.92	0.90	1.47	1.84
				1941 data				
		High nutrient				Low nutrient		
Crops	0.91	0.99	1.05	1.19	0.84*	0.95	1.04	1.03†
Roots	1.12	0.98	0.82	0.99	0.87*	0.93	0.92	0.88
Roots + crowns	1.07	0.94	0.85	0.95	0.88*	0.93	0.90	0.89
Total 1940–41	0.95	0.98	1.05	1.21	0.87*	0.93	1.09	1.11‡

* One plant dead and several others with root disease at final harvest.
† Omitting fourth and fifth crops, ratio = 1.16 (see table V).
‡ Omitting fourth and fifth crops (1941), ratio = 1.19 (see table V).

but also exceeded the yields of the medium-low sulphur plots and even equalled the yields of the medium sulphur and high sulphur plots. Analytical data to follow, indicate that these two crops received a nearly adequate supply of sulphur from some source other than the nutrient solution, possibly from the tar-covered walls of the sand bed, or from the sand itself. A careful examination of this container did not reveal any breaks in the tar lining or any other unusual appearance. It is difficult to understand how there could have been a sudden increase in the sulphur supply from the sand. No leaks were found in the system and the nutrient solutions did not show an appreciable increase in sulphate content.

In 1940–1941, as in 1939, the fumigated low sulphur and medium-low sulphur plots gave yields that were intermediate between the corresponding check yields and the largest yields obtained with adequate sulphur in the nutrient solution. This suggests that sulphur dioxide, supplied to the leaves for sulphur nutrition, is not as efficient as sulphate supplied to the roots. Presumably, this is due in part to the fact that sulphur dioxide, after absorption and oxidation to sulphate in the leaves, tends to remain in the leaves and is only slowly translocated to other parts of the plant.

The attempt was made in 1940–1941 to prevent the absorption by the sand of sulphur dioxide which would subsequently be oxidized to sulphate and absorbed by the roots. For this purpose, a stream of air (0.4 cubic ft. per minute) was forced upward through the sand throughout the fumi-
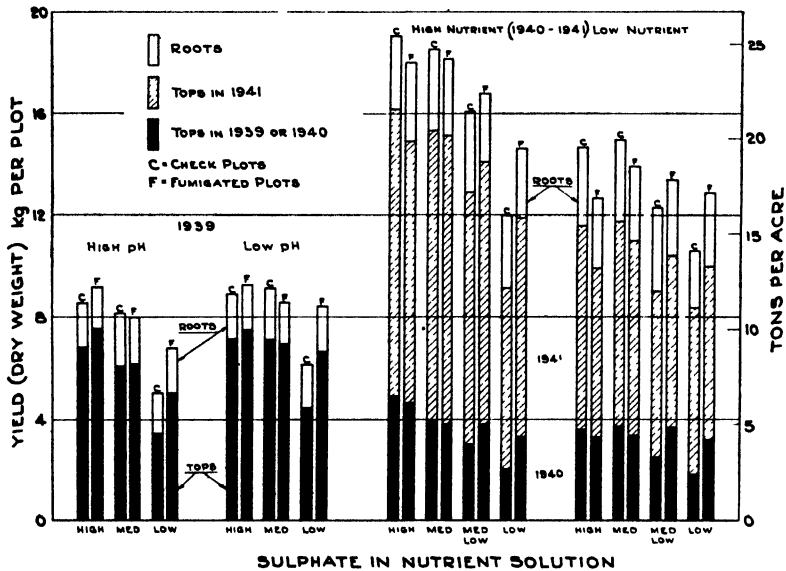
FIG. 3. Total yearly yields of alfalfa, including roots, for 1939, 1940, and 1941.

gation period. The check plots were similarly treated. This procedure was not adopted in 1939. Absorption of sulphur dioxide by the sand was never entirely excluded. Analysis of the nutrient solution indicated that its sulphur content was probably raised about 0.1 p.p.m. by this means in 1940–1941, but this increase seemed to have very little effect on the experiment.

The yield data suggest that the low sulphur and medium-low sulphur plots were much less deficient in sulphur in 1941 than in 1939 or 1940. In fact the yield of the large first crop in 1941 was nearly independent of the nominal sulphur supply. This was probably due to the long period during the winter when the plants could accumulate an appreciable amount of sulphur by slow absorption from the walls or elsewhere and also to the large root system in 1941. Further, it was impossible to operate the air-

washing system during the cold weather, and some sulphur was doubtless derived from the atmosphere at this time.

It will be noted that the weights of the different root systems did not parallel the variations in weight of the tops. In 1939 the medium sulphur check plots had slightly larger roots than the others; but the low sulphur roots were a little larger than the high sulphur roots. In all cases in 1939, except the low pH-high sulphur pair, the check roots were as large as or larger than the corresponding fumigated roots. However, the roots plus crowns were heavier in the check plots in only three of the 6 pairs. In 1941, the check roots and also roots plus crowns, exceeded the corresponding fumigated values in 7 out of 8 cases.

The ratios of yields of corresponding fumigated and check plots are summarized for all 3 years in table VI, and the yearly yields are shown graphically in figure 3. There was an increase in yield due to fumigation with sulphur dioxide in the low sulphur plots of 48 per cent. and 51 per cent. in 1939, and of 65 per cent. and 84 per cent. in 1940. The increase in the medium-low sulphur plots in 1940 was 25 per cent. and 47 per cent. The corresponding increases in 1941 were only 3 per cent. to 19 per cent., though as indicated in table VI, the 3 per cent. value was low due to the fact that the check plot of the pair which gave this result was somehow supplied with an adequate amount of sulphur on the fourth and fifth crops, as shown by analysis. Statistically, all of these increases in yield are highly significant.

The medium sulphur and high sulphur plots had ratios fairly close to unity. The 1939 fumigated plots tended to give a little larger yield than their checks, while the corresponding 1940 and 1941 fumigated plots gave somewhat smaller yields. In order to evaluate the statistical significance of these ratios, it is desirable to group together all the high nutrient plots which had an adequate supply of sulphur. These include the 1939 plots, and there is no apparent reason to make a distinction between those with low or high pH. The average of 8 ratios for the crops—4 in 1939, 2 in 1940, and 2 in 1941—is 1.00 with a standard deviation of 0.06. The average of 6 ratios of the roots—4 in 1939 and 2 in 1941—is 0.95 ± 0.11. These values are not significantly different from unity by the test of FISHER's t. Finally, the average of 6 ratios of total yield, including roots—4 for 1939 and 2 for 1941—is 1.00 ± 0.05. If the individual pairs of crop yields of this group are subjected to the analysis of variance, no significant effect on yield due to fumigation is indicated.

It may, therefore, be concluded from these experiments that the yield of alfalfa growing in a sulphur-deficient medium will be increased by sulphur dioxide fumigations which do not cause appreciable leaf injury, but not increased to the level of non-deficient plants. On the other hand, such fumigations will have no measurable effect at all if the roots are adequately supplied with sulphur and the general nutritional level is adequate. This latter observation is in accord with the results of experiments with alfalfa

grown in soil, previously published (11), and also with similar experiments by KATZ and LEDINGHAM (5). SETTERSTROM, ZIMMERMAN, and CROCKER (8) arrived at similar conclusions, using different nutrient solutions, which supplied both full nutrient and deficient nutrient.

The low nutrient plots in 1940–1941 present a somewhat confused picture. On the one hand, considering the sulphur-deficient plots, the increment of growth of the two fumigated plots with low nutrient was as great as, or even greater than, the increment of the other two fumigated plots with high nutrient. On the other hand, the two low nutrient fumigated plots with adequate sulphate, showed a decreased yield of 7 and 13 per cent. as compared with their checks. Considering the variability of the ratios already discussed, the 7 per cent. reduction in yield may not be significant, and the 13 per cent. reduction occurred on a plot which had some root disease. The yields of these two fumigated plots were similarly low on the second crop in 1940 when the nutrient level was high suggesting that they were inherently poorer plots than their checks. No conclusion can safely be drawn from these limited data concerning the effect of these fumigation treatments on the yield.

It should be noted, however, that the fumigations were not without effect on the leaves. Due to the long continued absorption of sulphur dioxide, which accumulated in the leaves as sulphate, the older leaves became chlorotic and some were eventually shed. The first two crops in 1940 showed this effect to a negligible extent. Subsequent crops showed it appreciably, particularly those that grew slowly at the end of the season. On the medium sulphur-low nutrient fumigated plot, the average shedding of leaves for 7 crops after the second crop in 1940 was 5 per cent. and the average extent of advanced chlorotic markings at harvest was also 5 per cent. of the leaf area. All of the other fumigated plots had approximately these values, except the high sulphur-low nutrient plot which had 6 per cent. of shed leaves and 17 per cent. of advanced chlorotic markings. The check plots had only about 1 per cent. of shed and chlorotic leaves on the average. On the high nutrient plots the amount of leaf destruction evidently did not influence the yield appreciably. Whether these foliar effects would have more influence on the yeld of plants growing in a low nutrient solution than on the yield in a high nutrient solution, needs further study.

PHOTOSYNTHESIS AND RESPIRATION

Two carbon dioxide autometers (10) were operated nearly continuously on some of these alfalfa plots in 1940–1941. Each machine sampled from the intakes and outlets of two plots, with a periodic and automatic interchange of sample sources so that an accurate comparison of the carbon dioxide exchange of the two plots was obtained. One machine was operated on the high sulphur plots; the other on the low sulphur plots. One fumigated plot and its unfumigated check were compared for two days, then the other fumigated plot and its check were measured for two days. Observations on

each crop were commenced as soon as the new growth had developed enough to give measurable activity. The data were calculated and plotted as previously described (10). Curves were obtained that were quite similar in appearance to those published earlier. In view of the fact that complete records were not obtained on any of the plots, it was not practicable to calculate a carbon dioxide balance sheet as was done before (10, 11). Since these measurements, however, were taken intermittently during the time when most of the crop growth occurred, they may be considered to be representative of the whole growth period. Accordingly, comparisons of the

TABLE VII

RATIOS OF NET ASSIMILATION AND YIELD ON THE FUMIGATED PLOTS TO THE CORRESPONDING VALUES ON THE CHECK PLOTS

CROP	HIGH S HIGH NUTRIENT		HIGH S LOW NUTRIENT		LOW S HIGH NUTRIENT		LOW S LOW NUTRIENT	
	NET ASSIMILATION	YIELD	NET ASSIMILATION	YIELD	NET ASSIMILATION	YIELD	NET ASSIMILATION	YIELD
				1940				
1st		1.15		0.75		1.54	1.37	2.25
2nd		0.88		0.94	1.32	1.18	1.44	1.83
3rd	0.95	0.99	1.01	0.93	1.26	1.70	1.44	1.81
4th	0.98	0.92	0.84	0.92	1.33	1.97	1.29	1.86
				1941				
1st	1.03	0.97	0.90	0.81	1.03	1.09	0.98	1.11
2nd	0.96	0.88	0.97	0.88	1.25	1.21	1.13	1.17
3rd	0.96	0.91	1.00	0.91	1.34	1.31	1.19	1.25
4th	0.95	0.86	0.83	0.79*	1.29	1.30	0.90	0.76†
5th	0.97	0.94	0.68	0.70*	1.15	1.10	0.67	0.69†
				Averages 1940–1941				
Tops		0.92		0.86		1.29		1.20
Roots		1.07		0.88		0.95		0.89
Total	0.97	0.95	0.90	0.87*	1.23	1.21	1.13	1.11

* Root disease developed in fumigated plot during 1941.
† Check plot received sulphur from an unknown source.

net assimilation based on carbon dioxide exchange should parallel the yield data, particularly the total yield, including the roots.

Table VII gives the net assimilation of four fumigated plots divided in each case by the net assimilation of the corresponding check plots. Similar ratios for the yields are also given. Two pairs of plots were high sulphur; the other two low sulphur. In the high nutrient-high sulphur pair of plots the ratios of the net assimilation for the crops were generally larger than the ratios of the crop yields. This was probably due to the fact that the fumigated roots were somewhat larger than the check roots, and therefore a larger proportion of assimilated material went into the fumigated roots, as compared with the tops, thus reducing the ratio of the top weights. The reverse was true of both the low sulphur pairs, particularly in 1940, when

there was a large difference between the ratios of net assimilation and crop
yield, indicating that the check roots were receiving a larger proportion of
the assimilated material as compared with the tops, than was the case with
the fumigated plots. In 1941 these differences were hardly apparent. The
low-nutrient-high sulphur pair showed only small differences throughout.
The final comparisons between assimilation and total yield, including roots,
were very close in all four pairs of plots.

<div align="center">WATER REQUIREMENTS</div>

The amount of water used in these experiments was determined for each
plot by frequent readings of the gauges on the supply tanks and by careful
attention to the distilled water and solution volumes added and sample
volumes taken. The water loss was largely transpiration, but there was a
measurable amount of evaporation from the surface of the sand, as indicated
by the losses which occurred when the amount of vegetation was small.
That these losses were not large was due in part to the low water-holding
capacity of the sand and in part to the sheet-aluminum cover between the
rows of plants. The gauge readings showed that during the first few days
of each crop, when transpiration was probably negligible, the plots lost
about 1.5 to 4 kilograms of water per day. It is estimated, therefore, that
the total water loss from the surface of the sand did not exceed 800 kilo-
grams during each season, and possibly was not more than about 600
kilograms.

The transpiration data are summarized in table VIII, as kilograms of
water evaporated per plot. In addition, the amount of water evaporated
per unit of dry matter produced is included. The latter values are calcu-
lated in two ways: first, from the water losses as measured, and then from
these losses less 800 kilograms, to give a minimum value for true transpira-
tion per unit dry matter.

Except for the low sulphur plots, which transpired considerably less
water per plot than the others, there was a rather narrow range in the
amount of transpiration in each group of plots. Transpiration was not
affected by the pH range employed, but it was lowered by lowering the
nutrient concentration, except in the low sulphur plots, in which it was
independent of the nutrient concentration. It is unlikely that any of the
differences in transpiration per plot between check and fumigated pairs are
large enough to be significant, except possibly in the low and medium-low
sulphur pairs in 1939 and 1940. In the latter cases, the fumigated plots
transpired only 5 to 13 per cent. more water than their checks; whereas
they had 25 to 84 per cent. more top growth than the checks.

The amount of water transpired in producing unit weight of crops was
much higher in the first year crops (1939 and 1940) than in the second
year crops (1941). Rather close concordance was noted between the 1939
crops and the comparable high nutrient crops in 1940. There was a much
smaller difference, however, between the 1939 crops plus roots and the com-

TABLE VIII

WATER REQUIREMENTS OF THE 1939, 1940, 1941 CROPS

YEAR	HIGH pH 1939 OR HIGH NUTRIENT 1940–1941								LOW pH 1939 OR LOW NUTRIENT 1940–1941							
	HIGH S		MEDIUM S		MEDIUM LOW S		LOW S		HIGH S		MEDIUM S		MEDIUM LOW S		LOW S	
	CHECK	FUMIGATED	CHECK	FUMIGATED	CHECK	FUMIGATED	CHECK	FUMIGATED	CHECK	FUMIGATED	CHECK	FUMIGATED	CHECK	FUMIGATED	CHECK	FUMIGATED
Kilograms of water transpired per plot																
1939	2610	2720	2560	2620			2120	2290	2550	2610	2760	2620			2210	2490
1940	1980	1970	1960	1850	1810	1930	1660	1740	1790	1880	1800	1780	1690	1820	1600	1760
1941	2640	2760	2890	2780	2980	2830	2210	2000	2430	2310	2430	2340	2300	2490	2320	2320
Kilograms of water per kilogram of crops (dry weight)																
1939*	383	360	422	410			625	456	359	349	389	378			501	375
1939†	266	255	290	295			388	277	246	242	276	263			320	254
1940*	406	423	443	433	600	515	832	529	493	565	480	530	673	495	915	548
1940†	242	251	262	246	335	301	432	286	274	325	267	292	354	277	457	298
1941*	234	268	263	255	300	273	305	232	304	346	302	331	351	368	352	343
1941†	162	190	190	182	220	196	195	140	204	226	202	227	229	249	231	224
Kilograms of water per kilogram of crops plus roots																
1939*	304	297	315	314			422	338	289	283	301	304			359	295
1939†	211	210	216	227			262	220	198	196	214	212			230	200
1940–41*	242	262	261	255	297	283	320	256	286	329	282	311	325	321	328	317
1940–41†	200	218	218	211	246	235	254	201	232	266	228	253	259	262	270	254

* Calculated from the total water transpired.

† Calculated from the total water transpired less 800 kg., to correct for evaporation from surface of sand and give a minimum value for true transpiration.

TABLE IX

TOTAL SULPHUR IN THE TOPS AND ROOTS OF 1939 ALFALFA

CROP	HIGH pH						LOW pH					
	HIGH S		MEDIUM S		LOW S		HIGH S		MEDIUM S		LOW S	
	CHECK	FUMIGATED	CHECK	FUMIGATED	CHECK	FUMIGATED	CHECK	FUMIGATED	CHECK	FUMIGATED	CHECK	FUMIGATED
	%	%	%	%	%	%	%	%	%	%	%	%
1st	0.25	0.32	0.21	0.30	0.17	0.27	0.26	0.32	0.24	0.30	0.16	0.23
2nd	0.27	0.35	0.23	0.32	0.12	0.19	0.28	0.33	0.23	0.30	0.11	0.19
3rd	0.31	0.40	0.27	0.37	0.14	0.19	0.33	0.39	0.33	0.38	0.12	0.24
4th	0.29	0.45	0.25	0.45	0.12	0.25	0.33	0.43	0.32	0.43	0.11	0.27
Weighted mean of crops	0.28	0.37	0.24	0.35	0.13	0.22	0.30	0.36	0.28	0.35	0.12	0.23
Weighted mean of leaves	0.60	0.85	0.62	0.86	0.26	0.53	0.60	0.88	0.53	0.86	0.26	0.49
Crowns	0.22	0.21	0.14	0.15	0.10	0.11	0.21	0.21	0.17	0.18	0.10	0.13
Roots	0.20	0.18	0.13	0.14	0.05	0.08	0.20	0.23	0.14	0.17	0.06	0.09
Hair roots	0.58	0.61	0.45	0.49	0.34	0.50	0.83	0.95	0.78	0.76	0.37	0.46
Weighted mean tops plus roots	0.28	0.35	0.23	0.32	0.13	0.20	0.30	0.36	0.28	0.34	0.13	0.22

parable high nutrient data for 1940–1941, indicating that the amount of water used in producing unit amount of total dry matter, including roots, was not greatly different in the first and second years of growth. As indicated later, this difference was eliminated completely when the water losses from the surface of the sand were considered.
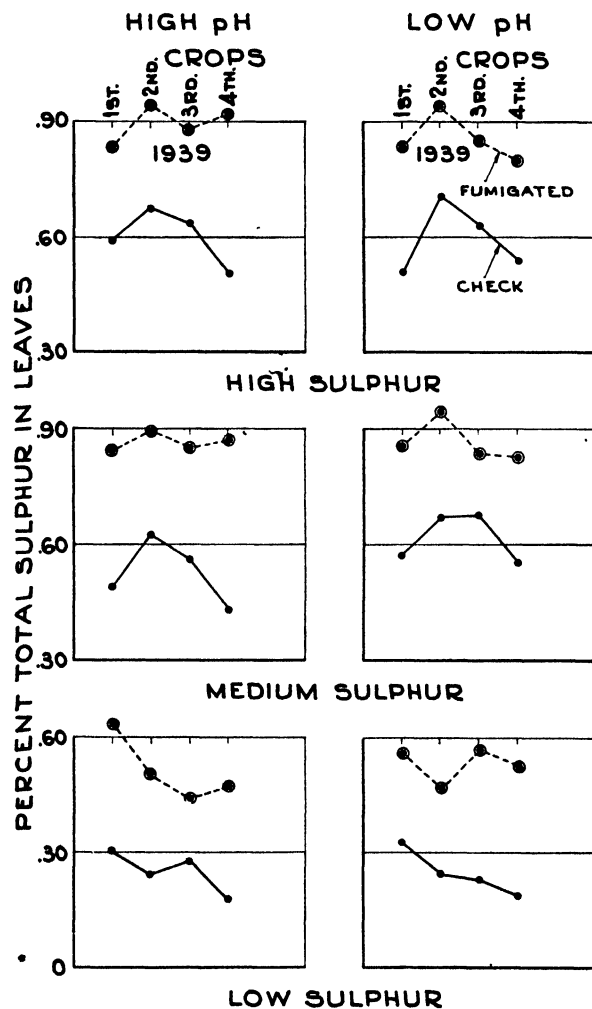
FIG. 4.   Sulphur content of the alfalfa leaves at the harvest of each crop in 1939.

Water transpired per unit of dry matter was appreciably higher at the low nutrient level than at the high, owing to the smaller crops on the former plots. Particularly large amounts of water were used by most of the check plots at low and medium-low sulphur levels, as compared with the fumigated plots. With medium and high sulphur there was no appreciable fumigation effect.

When allowance was made for the evaporation of water from the surface of the sand, the foregoing relationships were not materially changed

though some of the differences between the high and low nutrient levels disappeared, and the comparable high nutrient 1939 and 1940–1941 crops plus roots data became nearly identical. It should be noted that the allowance employed (800 kilograms per year) may have over-corrected for these losses, but a smaller allowance would merely have yielded larger transpiration values without affecting the other conclusions. Detailed data
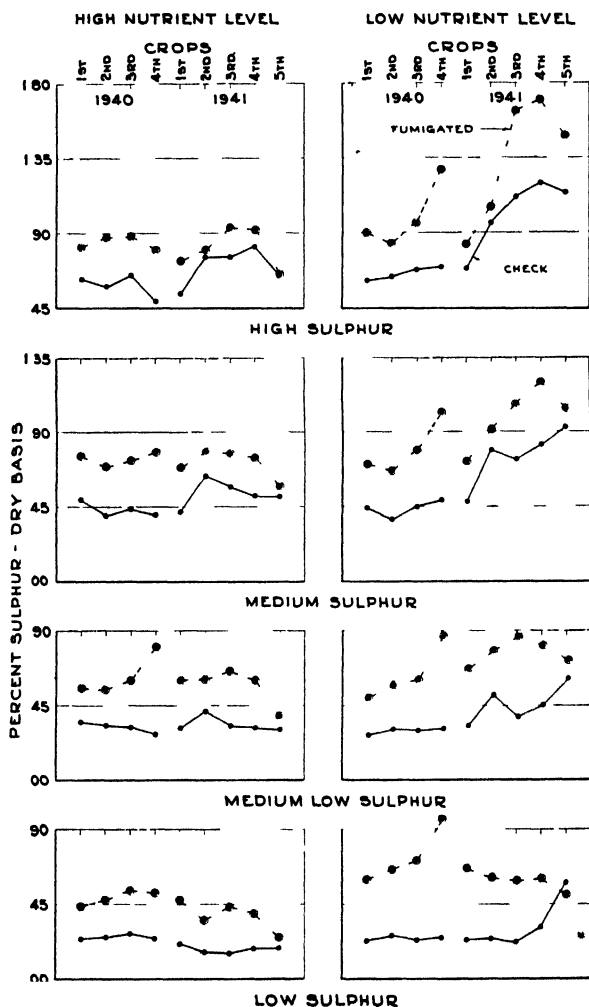
FIG. 5. Sulphur content of the alfalfa leaves at the harvest of each crop in 1940 and 1941.

for the different crops (not shown in table VIII) indicate that the water utilized in the summer was a great deal more than in the spring or autumn. For example, the value for an average first crop in 1941 was about 150 and for the corresponding fourth crop about 400 kilograms per kilogram of dry matter. This large divergence still remained after making allowance for evaporation from the surface of the sand.

TABLE X

TOTAL SULPHUR IN THE TOPS AND ROOTS OF 1940–1941 ALFALFA

CROP	HIGH NUTRIENT LEVEL								LOW NUTRIENT LEVEL							
	HIGH S		MEDIUM S		MEDIUM-LOW S		LOW S		HIGH S		MEDIUM S		MEDIUM-LOW S		LOW S	
	CHECK	FUMI-GATED	CHECK	FUMI-GATED	CHECK	FUMI-GATED	CHECK	FUMI-GATED	CHECK	FUMI-GATED	CHECK	FUMI-GATED	CHECK	FUMI-GATED	CHECK	FUMI-GATED
	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%
1940																
1st	0.26	0.40	0.22	0.31	0.15	0.23	0.13	0.24	0.39	0.44	0.29	0.38	0.24	0.34	0.16	0.23
2nd	0.27	0.37	0.21	0.29	0.18	0.25	0.15	0.24	0.33	0.41	0.20	0.30	0.20	0.32	0.16	0.29
3rd	0.34	0.41	0.21	0.33	0.17	0.29	0.16	0.25	0.34	0.44	0.24	0.38	0.17	0.29	0.19	0.30
4th	0.35	0.51	0.25	0.52	0.20	0.47	0.19	0.40	0.49	0.82	0.33	0.66	0.23	0.54	0.20	0.50
1941																
1st	0.24	0.28	0.18	0.28	0.16	0.24	0.10	0.21	0.32	0.39	0.25	0.31	0.18	0.27	0.11	0.27
2nd	0.37	0.38	0.30	0.35	0.21	0.29	0.09	0.17	0.43	0.48	0.35	0.41	0.27	0.38	0.13	0.27
3rd	0.35	0.40	0.24	0.31	0.16	0.25	0.10	0.18	0.48	0.60	0.30	0.39	0.18	0.32	0.12	0.21
4th	0.38	0.42	0.28	0.31	0.15	0.27	0.09	0.16	0.56	0.74	0.39	0.50	0.21	0.33	0.15	0.24
5th	0.40	0.39	0.32	0.31	0.19	0.24	0.12	0.15	0.70	1.10	0.57	0.60	0.45	0.48	0.37	0.32
Crowns	0.22	0.23	0.15	0.16	0.12	0.15	0.08	0.11	0.25	0.27	0.20	0.20	0.18	0.17	0.14	0.11
Roots	0.22	0.24	0.13	0.13	0.08	0.10	0.06	0.07	0.23	0.24	0.17	0.18	0.11	0.12	0.09	0.10
Hair roots	0.73	0.63	0.49	0.53	0.30	0.36	0.22	0.26	0.61	0.66	0.68	0.74	0.47	0.48	0.56	0.32
Weighted means 1940–1941 crops plus roots	0.33	0.39	0.24	0.31	0.16	0.26	0.11	0.20	0.41	0.52	0.31	0.40	0.21	0.31	0.17	0.26

THE SULPHUR BALANCE SHEET

In order to establish definitely the minimum sulphate concentrations in the nutrient solution necessary for adequate sulphur nutrition and also to determine the amount of sulphur dioxide absorbed in these experiments, it is desirable to make a comparison of the sulphur removed from the nutrient solutions with the amount found in the crops. This comparison should also furnish a criterion for evaluating the operation of the sand culture equipment.

The vegetation has been analyzed for sulphur by the Parr bomb. This simple method gives excellent results if the samples are allowed to stand for at least 6 days after precipitation before filtration. Tables IX and X summarize the total sulphur values for all the crops, including the roots. Figures 3 and 4 give the sulphur content of the leaves of all the crops. The data show an increasing amount of sulphur in the crops with increasing sulphur in the nutrient solutions. The data also indicate an increase due to fumigation with sulphur dioxide. Absorption of sulphur was apparently not influenced by the range of pH employed (table IX). There was a higher concentration of sulphur in the plant tissue at the low nutrient than at the high nutrient level (table X), and particularly in the leaves, as shown in figure 5. This difference was not caused by a difference in rate of absorption of sulphate but rather was due to the fact that the low nutrient plants were smaller than the comparable high nutrient plants. The high sulphur content of the hair roots is noteworthy. These values were about equal to the sulphur content of the leaves. In a majority of cases the check leaves had less sulphur than the hair roots, though the reverse was nearly always true of the fumigated leaves. The hair roots of the fumigated plants generally had more sulphur than those of the check plants. An important exception was the low sulphur-low nutrient pair, in which the high sulphur content of the hair roots of the check plot was associated with improved growth of the fourth and fifth crops as pointed out earlier.

The balance sheet of sulphur absorbed from the nutrient solutions and sulphur found in the 1939 and 1940–1941 crops, including the roots, is given in table XI. Check and fumigated plots are considered side by side. Decreasing sulphur additions are shown in the columns. The data show clearly that if sulphur was available either in solution as sulphate or as sulphur dioxide, it was readily absorbed by the plants. For an unknown reason, more sulphur was found in the plants of the check plots than was removed from the nutrient solutions. Evidently the check plants received sulphur from a source other than the nutrient solution. This sulphur is listed in the table as "unaccounted for."

It was presumed that the principal source of this sulphur was the tar lining the tanks, which contained about 3 to 4 per cent. sulphur, though long contact of distilled water with this tar has failed to dissolve more than a trace of sulphate. Since, however, the hair roots of the alfalfa were found to be thickly distributed over the tar surface, adhering firmly but

TABLE XI

BALANCE SHEET OF SULPHUR ABSORBED FROM THE NUTRIENT SOLUTIONS AND SULPHUR FOUND IN THE PLANTS

YEAR	TREATMENT		SULPHUR IN					
			CHECK PLOTS			FUMIGATED PLOTS		
			FROM SOLUTION	IN PLANTS	UNACCOUNTED FOR	FROM SOLUTION	FROM SO$_2$ (CALCULATED)	IN PLANTS
			grams S per plot					
1939	High pH	High S	22.8	24.3	1.5	19.0	12.0	32.5
		Medium S	16.9	18.9	2.0	12.8	11.0	25.8
		Low S	3.3	6.6	3.3	2.4	7.9	13.6
1939	Low pH	High S	25.0	26.8	1.8	23.2	8.3	33.3
		Medium S	22.3	25.7	3.4	18.3	7.3	29.0
		Low S	3.6	7.9	4.3	3.4	10.7	18.4
1940–1941	High nutrient	High S	56.6	62.7	6.1	46.3	14.2	66.3
		Medium S	29.5	42.9	13.4	24.8	17.0	55.2
		Medium-Low S	17.4	26.3	8.9	11.0	22.7	42.6
		Low S	1.5	12.9	11.4	1.1	14.9	27.4
1940–1941	Low nutrient	High S	53.3	61.5	8.2	40.3	19.2	67.7
		Medium S	31.0	47.8	16.8	17.0	21.9	55.7
		Medium-Low S	11.2	26.8	15.5	7.8	19.7	43.0
		Low S	2.4	20.3	17.8	1.7	14.9	34.4

not penetrating the layer, the possibility remained that the roots could have acquired sulphur directly from the tar in spite of its low solubility. For this reason, before the 1942 season started, the sand boxes were lined with plate glass, mounted in the tar. Only a few narrow seams of tar were left exposed, thus reducing the uncovered tar surface to a negligible area.

In spite of this extraneous source of sulphur, the balance sheet gives an excellent picture of the sulphur exchange. In every case the fumigated plots showed less absorption from the nutrient solution than the corresponding check plots. Evidently the additional supply through the leaves reduced the intake through the roots. The table gives an estimate of the amount of sulphur absorbed as sulphur dioxide on the assumption that the "unaccounted for" sulphur was the same in each fumigated plot as in its check. The amount of sulphur thus ascribed to sulphur dioxide was approximately the same in the different plots of each group, as would be expected from the fact that the fumigations were applied uniformly to all the treated plots. The "unaccounted for" sulphur was somewhat smaller in the high sulphur plots than with the other treatments, suggesting that this supply was not drawn on so heavily when there was adequate sulphate in solution.

Consideration of the values of the "unaccounted for" sulphur indicates that the low-sulphur check plots of 1940–1941 received a total amount of sulphur approximately equal to the amount accounted for as removed from the medium-low sulphur solutions. It is, therefore, probable that if the nutrient solution had been the sole source of the sulphur, the growth obtained on the low-sulphur plots could have been obtained with a solution about 0.8–1.0 p.p.m. sulphur, and the growth on the medium-low sulphur and medium sulphur, with concentrations 0.5–0.8 p.p.m. greater than those employed. Sulphur deficiency would then be expected with less than about 1.5 to 2.0 p.p.m. sulphate sulphur in the nutrient solution at the growth rates of these experiments.

Summary

Alfalfa has been grown in the large sand-culture equipment for three years. A wide range of concentrations of sulphate was employed in the nutrient solution, and one-half the plots were fumigated with 0.1 p.p.m. sulphur dioxide for about 7 hours each day and 6 days a week throughout nearly the whole life of each crop.

In 1939, 6 pairs of plots were studied using a nutrient solution in which the concentration of the principal nutrients was about one-half that of HOAGLAND's solution. Seedlings were started in the greenhouse and 4 large crops, including finally the roots, were harvested during the year. Three pairs of these plots were maintained at about pH 5.5; the others at about pH 7.0. Two pairs of nutrient solutions each had nominal sulphate concentrations corresponding to 0, 5, and 90 p.p.m. sulphur. The "zero" solution actually contained about 0.5 p.p.m.

In 1940 new seedlings were started for 8 pairs of plots. Four crops were harvested in 1940 and 5 crops, in addition to the roots, in 1941. A pH of about 5.5 was maintained throughout. Four pairs of plots had a nutrient solution of about 0.5 HOAGLAND's concentration; the other 4 pairs after starting at this strength, were progressively diluted until they reached 3 per cent. of the initial concentration. Two pairs of the nutrient solutions each had nominal sulphate concentrations of 0 (actually 0.2–0.3), 0.8, 1.5, and 10 p.p.m. sulphur.

The results of the experiments were as follows:

1. A pH of 5.5 was more favorable for growth of alfalfa than a pH of 7.0, with the lower sulphate concentrations. There was no pH effect with 90 p.p.m. sulphate sulphur in the nutrient solution.

2. The leaves of the sulphur-deficient plants were definitely chlorotic.

3. Yield of the sulphur-deficient plots was appreciably less than the yield of the plots with adequate sulphur.

4. Yield of the sulphur-deficient plots was improved by fumigation with sulphur dioxide. This source of sulphur was less efficient than sulphate for purposes of nutrition, at the concentrations applied.

5. Yield of the fumigated plots which had adequate amounts of sulphate in the nutrient solution was statistically the same as the yield of the unfumigated checks.

6. The root systems of the plots did not vary as greatly in weight as did the crops. In 1941, the check roots were heavier than the corresponding fumigated roots in seven out of eight pairs.

7. Net assimilation data from carbon dioxide exchange measurements confirmed the yield data.

8. Transpiration values, calculated from the water losses from the supply tanks, fell within a rather narrow range in each experiment, except that the low sulphur plots gave appreciably lower results. There was no discernible effect due to fumigation in the plots with adequate sulphur. In 1939 and 1940 the low and medium-low fumigated plots transpired only 5 to 12 per cent. more water than their checks, though they had 25 to 84 per cent. more top growth.

9. The water transpired per unit of top growth was greater in first year 1939 and 1940 crops than in the second year 1941 crops, but the amount per unit of total growth including roots was about the same in all three years. Transpiration per unit of top growth was particularly high in the sulphur-deficient check plots., The sulphur dioxide fumigations had no appreciable effect on transpiration in the plots adequately supplied with sulphate sulphur. Summer crops transpired much more water than the spring and autumn crops. These relationships were not greatly changed when the transpiration values per unit of dry matter were corrected for water evaporated from the surface of the sand.

10. Sulphur analyses of the vegetation showed that absorption of this element was increased by increasing the sulphate concentration of the nu-

trient solution or by sulphur dioxide fumigation. Absorption was not influenced by the range of pH considered. Nearly the same amount of absorption of sulphur occurred in comparable plots at the low and high nutrient levels, but owing to slower growth of the low nutrient plants, the sulphur concentrations in them were greater than in the high nutrient plants.

11. The sulphur balance sheet indicated that the plants received some sulphur from a source other than the nutrient solution. The amount of the unaccounted-for sulphur was least in the high sulphur plots.

12. The fumigated plants absorbed less sulphur from the nutrient solution than did the check plants.

13. With less than 1 p.p.m. sulphate sulphur in the nutrient solution there was definite evidence of sulphur deficiency in the plants. Taking the "unaccounted for" sulphur into consideration, it appeared that deficiency symptoms could be expected under the conditions of these experiments with less than about 1.5 to 2.0 p.p.m. sulphate sulphur, if the nutrient solution were the only source of the sulphur.

LITERATURE CITED

1. ALWAY, F. J. A nutrient element slighted in agricultural research. Jour. Amer. Soc. Agron. **32**: 913–921. 1940.

2. ——————, MARCH, A. W., and METHLEY, W. J. Sufficiency of atmospheric sulfur for maximum crop yields. Proc. Soil Sci. Soc. Amer. **2**: 229–238. 1937.

3. HART, E. B., and PETERSON, W. H. Sulphur requirements of farm crops in relation to the soil and air supply. Wisconsin Agr. Exp. Sta. Research Bull. 14. pp. 21. 1911.

4. HOAGLAND, D. R. Optimum nutrient solutions for plants. Science n.s. **52**: 562–564. 1920.

5. KATZ, MORRIS, and LEDINGHAM, G. A. Effect of sulphur dioxide on vegetation. Chapter XIII. Experiments on yield of barley and alfalfa. National Research Council of Canada. N.R.C. no. 815. pp. 447. 1939.

6. POWERS, W. L. Sulphur in relation to soil fertility. Oregon Agr. Exp. Sta. Bull. 199. 1923.

7. SCHERTZ, F. M. The extraction and separation of chlorophyll ($a + \beta$) carotin and xanthophyll in fresh green leaves, preliminary to their quantitative determination. Plant Physiol. **3**: 211–216. 1928.

8. SETTERSTROM, CARL, ZIMMERMAN, P. W., and CROCKER, WILLIAM. Effect of low concentrations of sulphur dioxide on yield of alfalfa and Cruciferae. Contrib. Boyce Thompson Inst. 9: 179–198. 1938.

9. THOMAS, MOYER D., HENDRICKS, RUSSEL H., IVIE, JAMES O., and HILL, GEO. R. An installation of large sand-culture beds surmounted by individual air-conditioned greenhouses. Plant Physiol. 18: 334–344. 1943.

10. ——————, and HILL, GEO. R. Relation of sulphur dioxide in the atmosphere to photosynthesis and respiration of alfalfa. Plant Physiol. 12: 309–383. 1937.

11. ——————, and ——————. The continuous measurement of photosynthesis, respiration, and transpiration of alfalfa and wheat growing under field conditions. Plant Physiol. 12: 285–307. 1937.

POLARIZATION AND STIMULATION OF THE ONION ROOT BY DIRECT CURRENT

L. Joe Berry and Rosalie C. Hoyt

(WITH TEN FIGURES)

Introduction

Previous work has shown (1) that the passage of a constant, positive electric current through an onion root (a) increased the positivity of the observed[1] electric potential when the current was sent up the root (polarization) and (b) decreased the observed potential when the current was sent down the root (depolarization). This confirmed earlier observations by Marsh (11) and was in agreement with similar experiments by Wilks (19) and Clark (8) with the Avena coleoptile, Rosene (17) with the Douglas fir, and Rehm (15) with *Phaseolus multiflorus*. This effect was due to changes within the root and not at the contacts. The magnitude of the change was greater when the potential was decreased than when it was increased. Moreover, basal segments of the root exhibited smaller responses to a given current flow than apical segments. Both hydrogen cyanide and hydrogen reduced the inherent electric potential to essentially zero and at the same time produced little, if any, change in the action of applied current. These effects were reversible. The minimum current required for producing (a) or (b) was well within the range of current magnitude that could be maintained by the root in an external circuit. It was higher in basal segments than in apical segments. With constant current applied for increasing time periods, the effect reached a maximum. These results were interpreted as being due to an ionic accumulation at "phase boundaries" within the cell. This potential due to polarization was shown to be independent of inherent potential. Several "anomalous" curves (1, fig. 9) were obtained, however, in which there was a decrease with upward current in contrast to (a) above, and it was thought that the current acted as a stimulus for the root with a resultant negativity in inherent P.D. similar to an effect produced by Marsh (12) mechanically in the onion root. Rehm (15) also found such a change but only when the applied current was several fold larger than that required for polarization. In a series of studies on the effect of applied direct current on the potentials of the coenocytic algae, Blinks (4, 5, 6) found that currents opposing the inherent flow increased the P.D. while series currents decreased the P.D. He was able, however, to show that with increased opposing current a stimulated condition could be produced in which the potential decreased rather than increased. The ease with which this effect could be obtained was subject to the previous history of the cells, particularly the ionic constituents of the medium in which they were kept.

[1] The usual convention is to measure the electric potential of the distal contact with respect to the earthed proximal contact.

In the present report, data will be given which will throw additional light on the changes produced in the polarity potential of the root of *Allium cepa* by small applied currents and which confirm earlier observations. It is believed that they show: (a), an increased magnitude of applied current may stimulate[2] rather than polarize the root and when this occurs the effect is reversible; (b), the apical segment of the root is more readily stimulated than more basal segments of the root; (c), the size of the response to a given amount of stimulating current is reversibly dependent on the time of application of current and may even appear as a polarization if time is sufficiently long; (d), the magnitude of current required to stimulate rather than polarize a root varies from root to root and seems to be less at lower temperatures; (e), a stimulated root is still polarizable; (f), a root whose inherent potential is lowered by the removal of oxygen can no longer be stimulated by a current which stimulated the root in air but is polarized by the same current; (g), there is a quantitative difference between polarization and depolarization and also a difference in magnitude of these two types of response in apical and basal segments; (h), the effects of current flow are relatively independent of the age and length of the root; and (i), the "electrodes" within the root responsible for polarization and for stimulation are to a large degree different and independent of one another.

Method

The apparatus used for this investigation was patterned after that described in earlier papers (1, 2) in which the arrangement of the electrodes and contacts within the experimental chamber and the electrical circuit and switching diagram are described. The potential measuring instrument was an RCA Ultrasensitive DC Meter with a sensitivity of two millivolts per scale division and could be read to an accuracy of one-fourth division. The period of the instrument was three seconds and its stability was excellent. Current was measured with a Leeds and Northrop type 2420C galvanometer calibrated so that one scale division equalled 0.10 microampere.

Roots were grown as usual in aerated tap water in an aquarium with the onions supported in flanged glass tubes which were held in paraffined wooden slats. All roots but one were trimmed from the bulb before it was transferred to the experimental chamber. The length of individual roots used in different experiments varied from 30 to 55 mm. and their diameter from 0.53 to 0.72 mm. Contacts were moved into place around the root and a thirty-minute period of equilibration was allowed before beginning the ex-

[2] For convenience of terminology, *polarization* will be used throughout this paper to signify an increase in positivity of the observed potential when current is sent up the root; *depolarization* will signify a decrease in positivity of potential when current is sent down the root; and *stimulation* will signify a change in potential contrary to these. These definitions are in good usage as long as the distal contact is positive to the proximal contact but become contradictory when the inherent P.D. is reversed. It is felt that the inaccuracies resulting from these arbitrary definitions will be less confusing to the reader than strict adherence to the usual meaning of the words.

periment.   Growth was recorded at thirty-minute intervals throughout the
duration of an experiment with the aid of an ocular micrometer in a hori-
zontal microscope.   The root was always returned to the aquarium at the
end of a test and its growth again measured the following day.   Failure to
grow was taken as evidence of injury and three experiments were discarded
for this reason.   Electrodes of zinc-amalgam zinc sulphate were used.   Pre-
vious work using separate contacts for applying current (1) has shown that
the effect of current flow is on the root segment between the contacts and
not at the contacts themselves.   Therefore, in the experiments reported here
the same contacts were employed both for applying current and measuring
potential.   In this way the extreme tip of the root could be included in the
P.D. measurements.   The electrodes were checked at the beginning and end
of each run to assure their isoelectric and non-polarizable condition.   An
upper glass ring and lower glass cup were used as contacts on the root and
contained the tap water medium in which the roots were grown.

To change temperature during an experiment, water in a bath was cir-
culated by means of a small centrifugal pump through a double walled cop-
per experimental chamber.   The front and back of this chamber had glass
windows and the outer wall was heavily paraffined to reduce heat exchange
with the surroundings.   The water was cooled with ice and the temperature
was regulated manually to within 1° C.   A thermometer extending into the
chamber with the bulb just above the onion was used to follow the tempera-
ture.   Room temperature varied between 18° and 29° C. during the course
of the investigation.   This range proved to be of great value in revealing the
importance of temperature in determining the type of response elicited by
current; i.e., polarization or stimulation.   Control experiments show that
the electrodes undergo a considerable change in conductivity but remain
isoelectric over the temperature range used.

Cylinders of commercial oxygen and hydrogen were used as a source of
these gases.   The gases were saturated with water vapor by sending them
through gas washing towers before passing them into the experimental
chamber.

The strength, duration, and direction of current flow through the root
was varied from experiment to experiment and at times within a given ex-
periment.   During the application of current, no potential measurement
was made but the switch from the root to the Sensitive Meter was turned to
the "on" position at the same time the current was turned off.   A reading
was made within three seconds and at 15-second intervals for the first minute
thereafter.   Subsequent readings were made at 30-second intervals.   Some
points were omitted in redrawing the curves for publication if no change in
the shape of the curve resulted.

Since the pathways of current flow through a root are not known, the
current density may not be uniform over a given cross-sectional area and
may vary along the root axis.   For this reason, it is felt that current densi-
ties have an unknown significance and current magnitudes are therefore
given only in microamperes.

Procedures and results

EFFECT OF CURRENT OF VARIABLE MAGNITUDE AND CONSTANT DURATION ON THE ELECTRIC POTENTIAL WHEN SENT ALTERNATELY UP AND DOWN THE ROOT

With contacts placed at 0 and 5 millimeters above the root tip, current of varying strength was sent alternately up and then down the root for 30
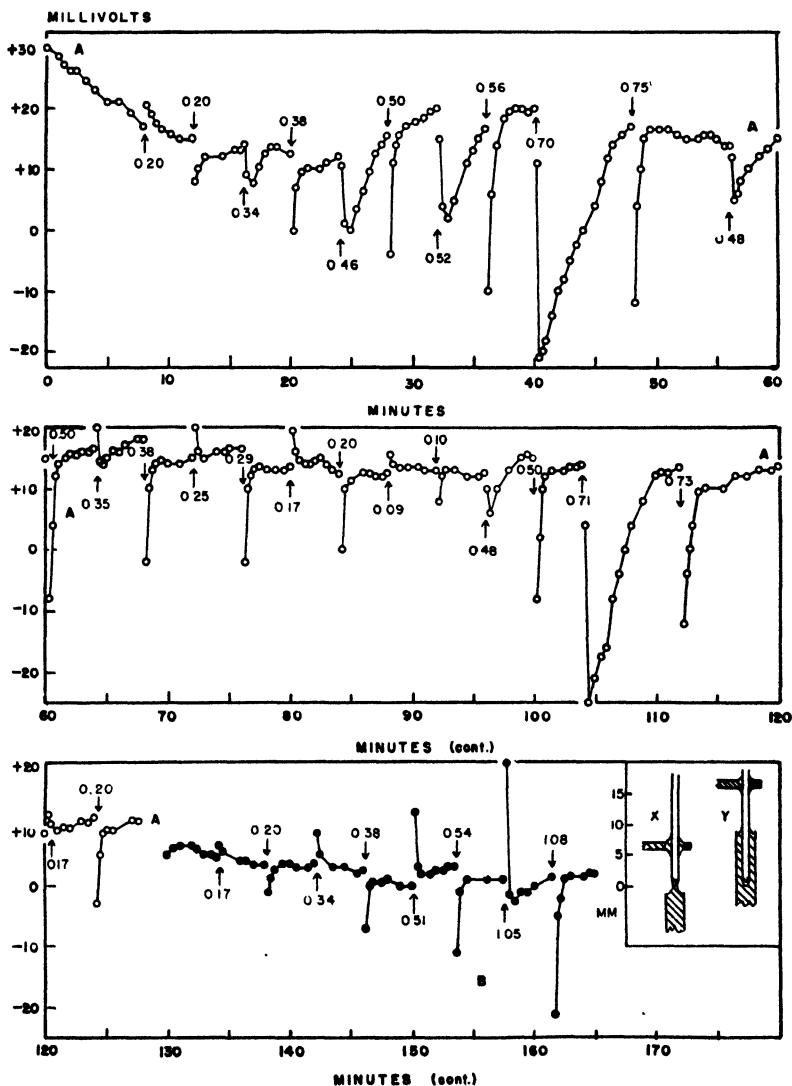
FIG. 1. The effect of applied current of variable magnitude on the inherent potential of the onion root. Arrows pointing upward indicate positive current sent up the root, arrows pointing downward indicate positive current sent down the root. The number with each arrow indicates the magnitude of the applied current in microamperes. In curve A the contacts were at 0 and 5 mm. and in curve B they were at 5 and 10 mm. on the same root, as shown in insets X and Y. The time of current flow was 30 seconds.

seconds and the change in potential was followed. The periods between the application of current were four minutes for the lower magnitudes and eight minutes for the higher magnitudes. Curve A, figure 1, is typical of the results obtained. Inset X, figure 1, shows the position of the contacts on the root. Upward current when sufficiently small (0.20 microampere) polarized the root but as the magnitude was increased in small increments to 0.70 microampere, it stimulated the root. With a reduction in current, a return to the polarizing effect showed almost a perfect reversibility. This reversibility was further emphasized by the subsequent stimulating and then polarizing action of the current as it was again increased and decreased. When current was sent down the root, only·depolarizations were produced and the magnitude of the potential change was dependent, at least in part, on the strength of the current. However, 0.56 microampere depolarized the root almost as much as 0.75 microampere. Attention is called to the fact that the decrease in inherent potential is greater when the root is stimulated than it is when depolarized even though in both cases a negative potential is initially observed. It must also be emphasized that the absolute magnitude of current necessary for stimulating the root is different for different roots and temperatures. The contacts were raised on the same root to 10 and 15 millimeters above the tip, inset Y, figure 1, and curve B (fig. 1) was obtained. All time intervals between current flow were four minutes. This more basal segment was not stimulated with upward current even though 1.05 microamperes were used; but there is an indication of slight stimulation, as shown by the small downward cusp, when the maximum current passed up the root. This type of response, however, is more evident in other curves.

A comparison of the magnitude of polarization with depolarization when currents of comparable strength were used in the apical segment verify an earlier observation (1) that the former is less than the latter even though in the earlier observation the absolute magnitude of current as well as time of application were different. The same relation to a less marked degree holds for the basal segment. It is also seen that the amount of depolarization, particularly, is less for a given current in a basal segment than in an apical segment (1).

A COMPARISON OF THE EFFECT OF CURRENT ON VARIOUS SEGMENTS OF THE ROOT

To further emphasize the differential behavior of a polar system to the same current, four five-millimeter segments of the same root (0 to 5 mm., 3 to 8 mm., 5 to 10 mm., and 10 to 15 mm.) were subjected to the same current successively. Insets W, X, Y, and Z in figure 2 show the positions of the contacts on the root that gave curves A, B, C, and D, respectively. Curve A' was obtained by returning the contacts to position W. The current was sent alternately up and then down the root with eight minutes between each application. With this particular root, 2.00 microamperes for 30 seconds were required for a stimulating current (curves A and A') but

only the first five millimeters of the root showed this 'type of response while the second segment gave some indication of being only partially stimulated (curve B). The two more basal segments, like the one in figure 1, were only polarized and depolarized (curves C and D).

The depolarization was progressively less as more basal segments were used if curve A′ is taken as typical for the apex. The failure of curve A
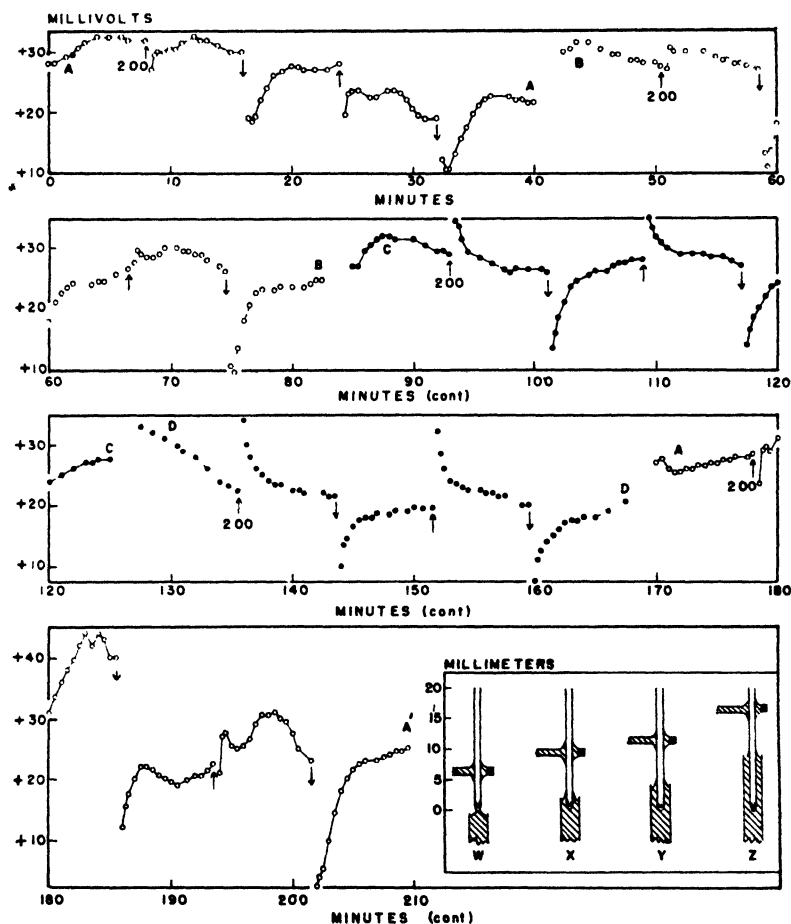
FIG. 2. A comparison of the effect of a stimulating current on various segments of the root. The direction of the arrows indicates the direction of applied current. The magnitude of the current was 2.00 microamperes and the duration of flow was 30 seconds. The positions of the contacts on the same root were: curve A, 0 and 5 mm.; curve B, 3 and 8 mm.; curve C, 5 and 10 mm.; curve D, 10 and 15 mm.; curve A′, 0 and 5 mm. (insets W, X, Y, Z).

to show this characteristic suggests that a partial stimulation occurred with downward current but its effect was masked by the depolarization imposed on it. This of course assumes that when the contacts were returned to position W, the proximal cells of the segment were less easily stimulated than originally and therefore the depolarizations in curve A′ were greater than

those in curve A. Evidence for such a suggested change in irritability is to be found in the work with alternating current (3). Polarizations were again less than depolarizations except in D, where they were essentially equal. As shown in curve 9, this seems to be somewhat characteristic of more basal segments.

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EFFECT OF CURRENT OF CONSTANT MAGNITUDE AND VARIABLE TIME ON
THE OBSERVED POTENTIAL OF DIFFERENT SEGMENTS OF THE ROOT

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Curve A of figure 3 was found by placing the contacts at 0 and 5 millimeters above the tip of the root, inset X, figure 3. Curve B and inset Y of figure 3 show the result with a segment 5 and 10 millimeters above the tip of a second root. The time scale refers to the absolute value only for curve A with zero time beginning at 120 minutes for B. The time interval between application of current in all curves was 8 minutes.

Opposing current of 0.40 microampere was sufficient to stimulate the root of curve A when applied for 5 seconds. There was no appreciable change in the magnitude of the decrease in inherent potential when the periods of application were 10, 15, and 30 seconds. When current was sent up the root for one minute, however, the initial potential was the same after current flow as before but there was a subsequent drop and recovery which gave evidence of a stimulated condition. Increasing the current to 1.00 microampere with the same root reveals that the maximum decrease in P.D. as a result of stimulation was obtained within 5 seconds and that it became less as the duration of current flow was increased to 10 seconds. For 30, 60, and 120 seconds there was an initial polarization but with a subsequent total fall in potential much below the initial value. Recovery was almost complete after the 30-second application but was obviously incomplete after the two longest periods. There is a reversal of electrical polarity during this time and current sent up the root *decreases* the P.D. to about zero but increases the positivity of the apical contact (see footnote 2). This root was still capable of being stimulated even though the inherent potential was negative when current passed up the root for 15 seconds. Time periods as short as it was possible to work the switches (not shown on curves), failed to give any indication that there was an initial polarization preceding stimulation similar to that found by BLINKS (5, 6). In his technique it was possible to apply current and make potential readings simultaneously. It is apparent from this curve that a stimulated region is still polarizable and that the observed potential change following applied current is an apparent algebraic summation of two oppositely oriented changes induced in the system. The response to stimulation must be the more rapid change of the two since it is the first to appear.

Since BERRY (1) showed[3] the magnitude of polarization for a given cur-

[3] 0.50 microampere was the only current used in the earlier study except in determining the threshold of polarization. Similarly, the duration of current flow was kept at 60 seconds except for the investigation of the magnitude of polarization as affected by time. The choice of these two values was dictated by the results of MARSH (11) in which he found these values to be without injury and to render decisive results.

rent increased with time up to about one minute then, in this case, as the duration of current flow increased the change in potential due to polarization became greater and the observed effect of stimulation diminished. How-
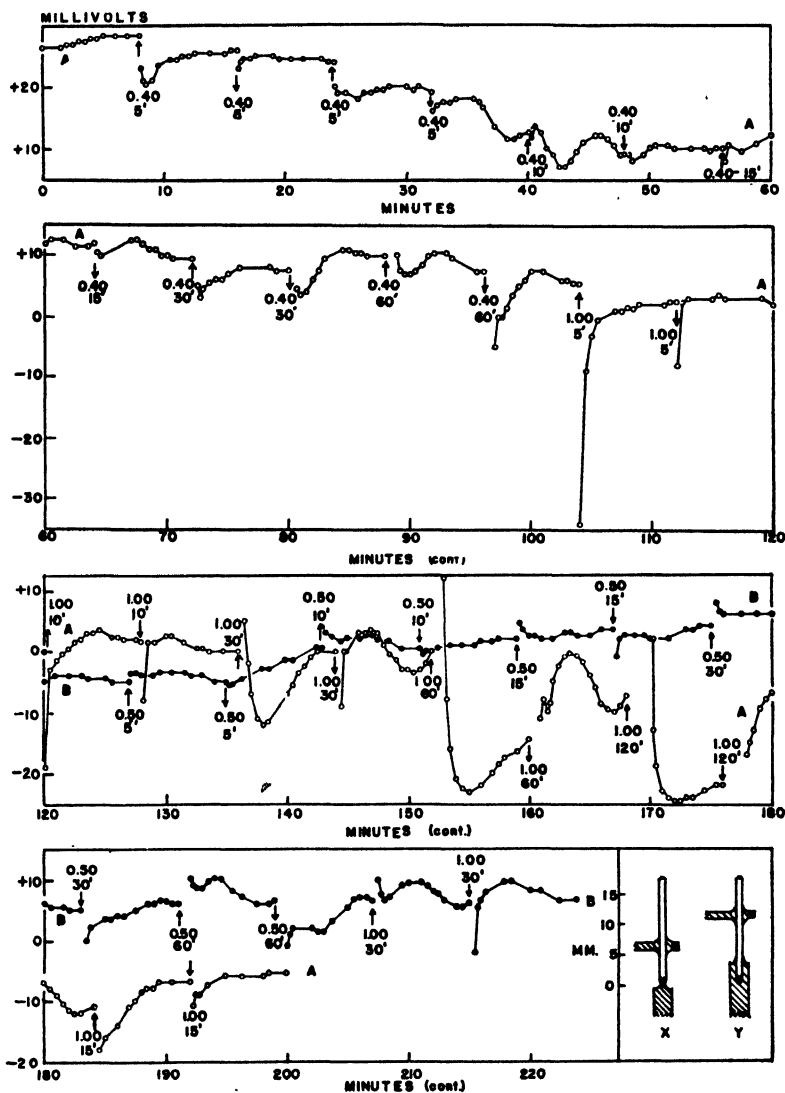
FIG. 3. The effect on inherent potential of current applied for varying lengths of time. The direction of the arrows indicates the direction of the applied current. The numbers with each arrow indicate the magnitude of current in microamperes, and the duration of current flow in seconds ('). The positions of the contacts were: curve A, 0 and 5 mm. (inset X); curve B, 5 and 10 mm. (inset Y). Curves A and B were obtained with different roots.

ever, these results may also be attributed in part to some type of change in the cells along the root axis in which the response to stimulation decreases with time of current flow. Such an effect has been found with AC. (3).

The similarity between the changes produced in curve A when 1.00 microampere was sent up the root for 60 and for 120 seconds, indicates that for a given set of conditions the effects due to stimulation and to polarization reach a maximum value and hence give a constant change. No doubt one of the causes for the earlier failure (1) to find more roots that were stimulated ("anomalies") may be attributed to the use of 60-second periods of application. In several curves, the resultant potential change after current flow was similar to that in curve B after 1.00 microampere was applied for 30 seconds. The behavior of the potential in curve B, figure 1, following upward current of 1.05 microamperes, mentioned above, is apparent.

Depolarizations of the root by 0.40 microampere increased as the duration of current flow was lengthened to 60 seconds. The shorter applications show some variations of small magnitude but differences from one depolarization to the next with constant conditions make these have doubtful significance. With 1.00 microampere, however, it was found that, contrary to the results when current passed up the root, the stimulating effect of downward current becomes more noticeable with longer time periods. The failure of the 15-second interval of flow to stimulate after the 120-second interval had stimulated the root shows that the effect was not necessarily due to some injury or abnormality. Moreover, the stimulation of a root with downward current suggests that the region in the root in which the current enters (in this case the upper contact) is the one more readily stimulated. This interpretation probably accounts for the failure to obtain an increasing depolarization with 1.00 microampere as the time of current flow increased from 5 to 30 seconds.

Curve B is typical of a segment of root in which no stimulation was apparent with the magnitude of current used. Potential alterations progressively increased with time and were in accord with earlier results (1).

EFFECT OF DECREASING THE TIME BETWEEN APPLICATIONS OF CURRENT ON THE RESPONSE OF INHERENT POTENTIAL TO CURRENT FLOW

In the previous section, the result of increasing the time period of current flow through the root gave results which are interpreted as being due to a summation of oppositely oriented responses. The ability of a stimulated root to polarize is demonstrated in the present section. Curve A of figure 4 was obtained with the contacts at the apical 5 millimeters, inset X. This particular root required 1.50 microamperes for stimulation as indicated by the first part of the curve. The duration of current flow in all cases was 15 seconds. The root was stimulated at 8-minute intervals and after the fourth time the same magnitude of current was sent up the root one and one-half minutes later and again after two minutes. Following an eight minute recovery period, the three applications of current with the short time intervals between were repeated and so on. In the first two cases both the first and the second application of current stimulated the root. The third application gave rise only to a polarization. In the rest of the series,

only the first of the current applications stimulated and the other two polarized the root. Thus, before recovery from stimulation is complete, it is possible to polarize the root with the same current. Failure of the two more
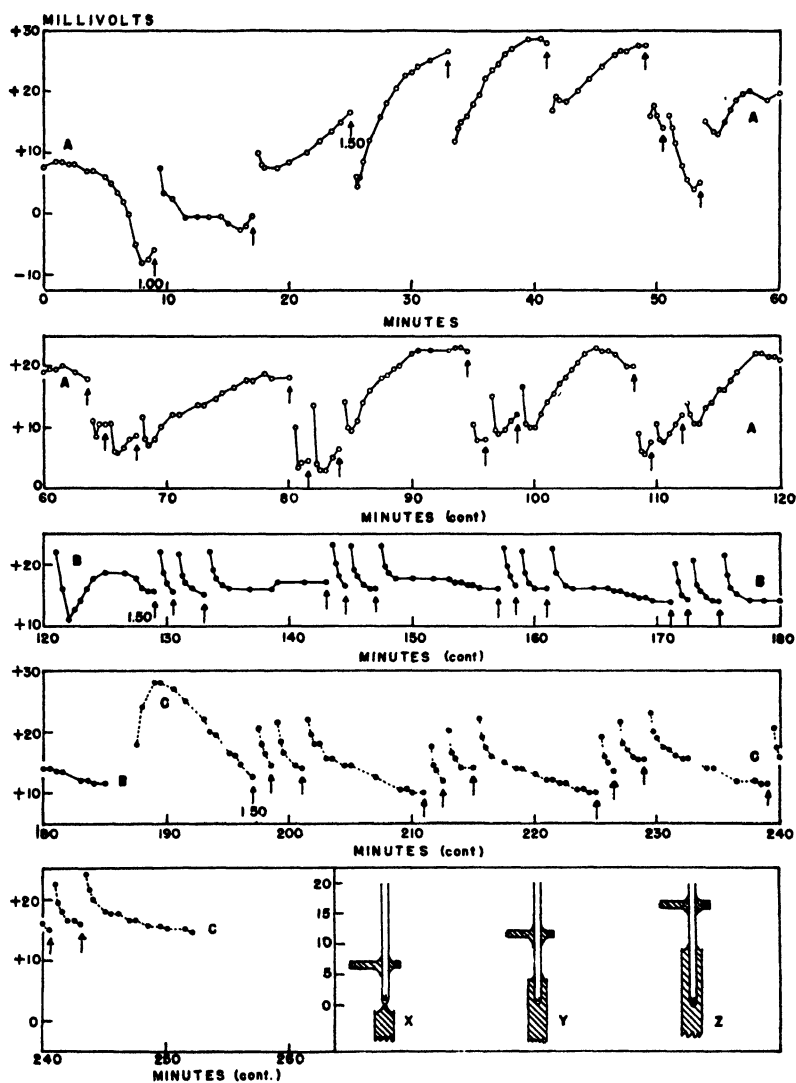
FIG. 4. The effect of decreasing the time between applications of current on the response of inherent potential to current flow. The direction of the arrows indicates the direction of the applied current. The numbers at the arrows give the magnitude of the current in microamperes. (Where no number is given, the current was the same as that last indicated.) The duration of current flow was 15 seconds. Curves A, B, and C were all obtained with the same root and the positions of the contacts were: Curve A, 0 and 5 mm.; curve B, 5 and 10 mm.; curve C, 10 and 15 mm. (insets X, Y, Z).

basal segments (5 and 10 millimeters, and 10 and 15 millimeters up the same root, insets Y and Z) to behave in the same way is shown in curves B and C, respectively (fig. 4). The level of polarization reached in B is

essentially the same for all applications of current.   In C, with each succes-
sive current flow in a series of three, there is an increase in the absolute
potential reached but the magnitude of polarization is approximately equal.

Figure 5 presents the results from an apical 5-millimeter segment of
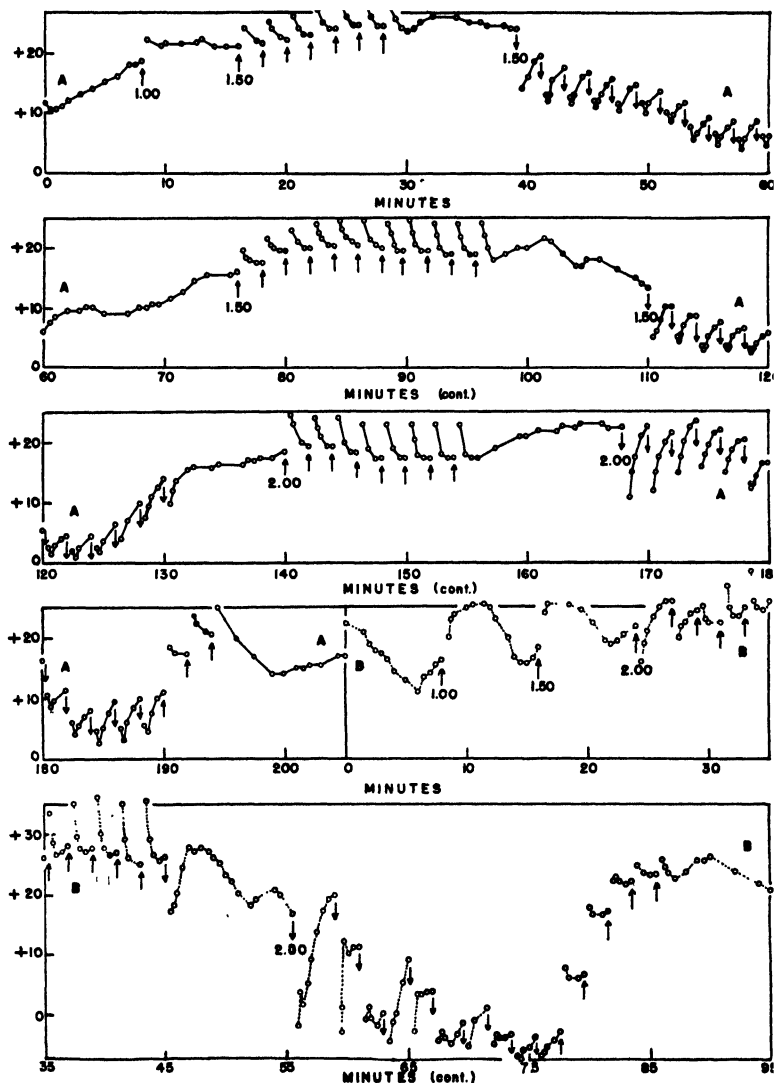
FIG. 5.   Same as figure 4.   Curves A and B were obtained from two separate roots
and the positions of the contacts were: curve A, 0 and 5 mm.; curve B, 0 and 5 mm.

root (contacts placed as shown by inset X, fig. 4) which gave only polariza-
tion or depolarization when current was sent either up or down the root
every two minutes several times in succession on the time scale.   The dura-
tion of flow was 30 seconds in each case and curve A is typical of the results
obtained.   Current of 1.50 microamperes sent up the root seven times be-

tween 16 and 30 minutes and eleven times between 76 and 96 minutes caused a progressively steeper and slightly larger polarization during the first five applications in each series and then produced an essentially constant polarization in the remaining applications. With the magnitude of current raised to 2.00 microamperes between 140 and 155 minutes and applied eight times, all polarizations were essentially alike. This suggests that the charges responsible may be built up gradually with weaker currents to a constant polarization while with stronger currents the initial application achieves a maximum effect. Since it has been shown (1) that increasing duration of flow raises polarization to a maximum, it would be expected that increasing the magnitude of current should have a similar effect.

Depolarizing current of 1.50 microamperes was applied eleven times between 40 and 60 minutes and again between 110 and 130 minutes. In the first series not only were smaller depolarizations produced but the type of potential change was altered as the electrical polarity decreased and then became practically constant. In the second series, however, a similar progression of changes was altered in the last three applications by a rather sharp rise in the inherent potential. There is no reason to think that polarizing or depolarizing currents are capable of causing any change in the spontaneous fluctuations in potential that exist in the absence of any known external stimuli (2), and hence may alter the *observed* effect of applied current. The series of depolarizations with 2.00 microamperes between 170 and 190 minutes is somewhat similar to the above. It should be noted that at the end of this series polarizing currents were applied three times and the first, as well as the second to a smaller degree, increased the potential without any appreciable fall to a lower value (see also curve B).

Roots capable of being stimulated were treated in a manner similar to the above and curve B, figure 5, is typical of the results obtained. The contacts were placed as shown in X, figure 4. 2.00 microamperes upward current stimulated the root but polarized it after the third application. The same intensity of current sent down the root at the end of this series produced a perfectly normal depolarization. Successive depolarizations produced changes similar to those in curve A but with less regularity in the sequence. Then the final passage of current up the root following the above was again effective in restoring the electrical polarity before normal polarizations were obtained.

The initial fall in potential immediately following the passage of current down the root that sometimes occurs before the rise in potential, appears in several cases in figure 5 (see also figs. 2, curve A; 6; 7; 10). The mechanism involved in this effect of current flow is not understood but the explanation that seems most likely on the basis of our present knowledge is that a small stimulation has been produced and summed with a depolarization. A more rapid recovery from stimulation must occur initially and thereby reduce the potential before recovery from depolarization restores the potential. A similar type of change following the passage of current up the root on the

other hand would depend upon polarization recovery occurring more rapidly than stimulation recovery. However, since AC (**3**) response may also have an initial downward cusp preceding the rise, a differential rate of recovery in apical and basal cells may be the whole basis of such changes in the potential; *i.e.*, in the absence of any but a stimulating action.

Effect of Temperature on the "Threshold" of Stimulation in Apical and Basal Segments of the Root

From observations of the effect of room temperature in summer and winter on the "threshold" of stimulation of roots, it was considered desirable to test experimentally the changes produced by temperature on a single root. The technical difficulties involved warrant a brief description. The circulation of cold water through the copper jacket used as a chamber for this part of the work caused a condensation of water vapor on the inner wall. This drying action gave rise to a shrinkage of the root as the temperature was lowered. On raising the temperature, there was a condensation of moisture on the root which lagged behind the chamber in warming. In order to avoid desiccation, the lower cup contact was raised around the root to a height of about 20 to 25 millimeters during the hour in which the temperature was first reduced. No shrinkage was observed during this time but growth was usually less than normal. A perfect equilibrium between water vapor and the air within the chamber was not reached since upon resuming the experiment at the end of this time slight shrinkage was observed. This shrinkage was not more than 0.30 millimeter for the entire period at the low temperature, and with the later increase in temperature normal growth was resumed. The check on growth the following day showed that only one root was damaged by this treatment.

In order to determine the effect of the shrinkage alone on "threshold" air at room temperature was dried by passing it over anhydrous calcium chloride and then circulating it through the chamber. An equivalent shrinkage was obtained. Of five roots thus treated, only one gave a lower "threshold" of stimulation while three had a higher "threshold." (There were no exceptions to the results with temperature.) With this evidence plus some checks in which roots were placed in test tubes of water and transferred to the refrigerator for several hours before use, it is considered certain that temperature has the effect described below. It is also true that the difference in the behavior of the same lot of onions in summer and winter is in accord with the other results.

In figure 6, the behavior of an apical five-millimeter segment of root to temperature and current is shown (inset X, figure 4). The current was passed through the root for 30 seconds each time and was alternated up and down. Both 1.50 and 2.00 microamperes stimulated the segment when sent up the root at room temperature (21°–22° C.). When the temperature was lowered to between 10° and 12° C., 1.00 microampere upward current partially stimulated the root and did stimulate it when passed downward. Both

1.50 and 2.00 gave greatly augmented responses when passed up the root but only the latter when passed down. 1.00 microampere again applied at intervals during the next hour stimulated the root when sent in either direction but particularly when sent upward. After an interval, as designated by the time scale, the temperature increased with a resultant fall in the ease of stimulation and during the final period at room temperature it was not
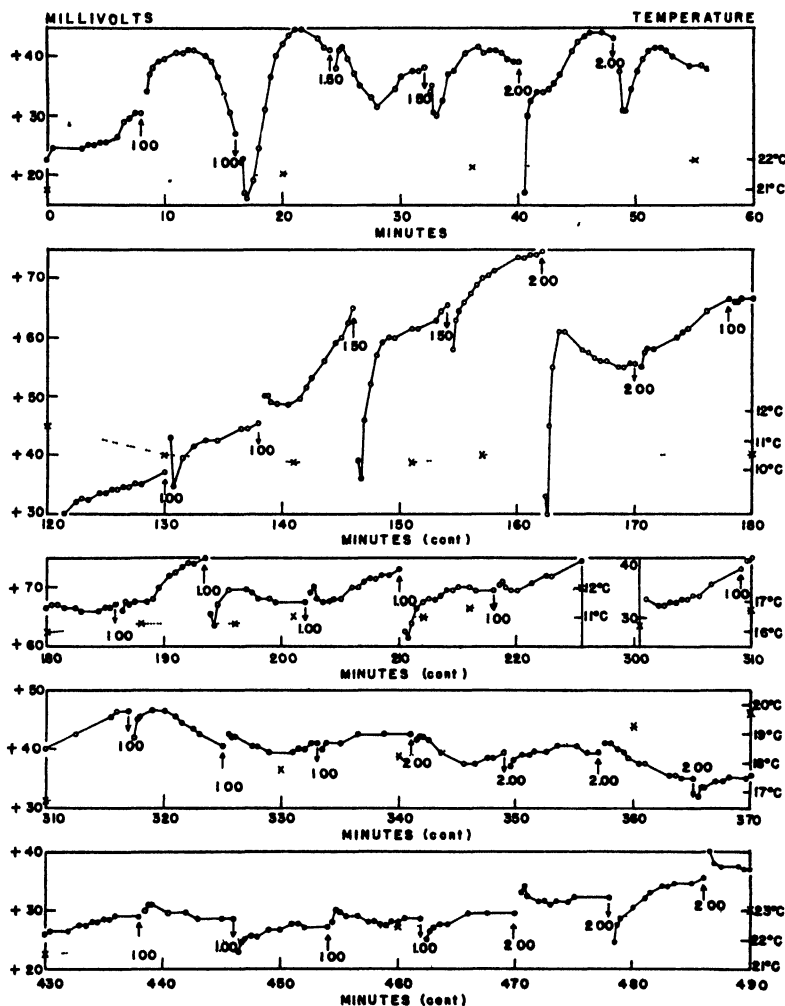
FIG. 6. The effect of temperature on the "threshold" of stimulation. The arrows indicate the direction of applied current up or down the root. The number with each arrow indicates the magnitude of the current in microamperes. The application time was 30 seconds, and the contacts were at 0 and 5 mm. The temperature scale is given on the right, and the variation of temperature with time is given by the broken curve.

possible to stimulate the root as easily as it was at the beginning. Attention is called to the abnormally high P.D. observed in this root at the lower temperatures. This is in contrast to the effect of temperature on potential

reported for the frog skin by LUND and MOORMAN (10) and for Valonia by MARSH (14). BLINKS (7), on the other hand, reports an increase in the potential of Valonia both at low and high temperatures with a minimum P.D. in between the extremes. With an onion root, however, the potential of which is measured in air and is subjected to the drying mentioned above,[4] it is unlikely that any real significance should be attached to the magnitude of P.D. observed at any temperature. As shown by ROSENE (16), the presence of water around a part of the root shunts the E.M.F. of these cells and adds or subtracts from the total electrical polarity of the system depending upon the orientation of the electrical gradient of the shunted segment. Since a slight surface film of water is normally present on the root, the change of humidity accompanying temperature alterations might alter the action of temperature alone on potential. The root represented in figure 6 is the only one of this series that exhibited such a marked alteration in inherent potential.

More typical of the response of the polarity potential to a lower temperature are the two curves in figure 7. Curve A is from the first 5 millimeters of the root tip with the contacts as diagrammed in inset X, figure 4. The initial temperature was between 24° and 25° C. and only polarizations and depolarizations were produced respectively when current of as much as 2.00 microamperes passed up or down the root. A decrease to between 12° and 13° C. found 0.50 microampere giving a very large stimulation as an opposing current but only a depolarization when in series. One microampere stimulated the root when sent in either direction. Results with 1.25 microamperes were not as clear. It is believed that the failure to obtain clear-cut stimulations may be attributed to current of too great an intensity polarizing or depolarizing the root after stimulation had occurred (compare fig. 3). A shorter application of current would probably have revealed a response similar to that in curve B below.

The effect of temperature on a basal segment is not as marked as on an apical segment [compare the AC results of BERRY and HOYT (3)]. Typical of this behavior is the 10- to 15-millimeter region (inset Z, fig. 4) that was used on several different roots like the one shown in curve B, figure 7. No stimulations were produced by the current passing in either direction at the higher temperatures. At the lower temperature, polarizations became progressively smaller as the current increased by increments of 0.50 from 1.00 to 2.00 microamperes. Partial stimulations of increasing magnitude were doubtless responsible. Note that this is contrary to the condition at both the warmer temperatures. All three downward applications of current at about 11° C. clearly caused a visible stimulation which was rendered obvious by decreasing the time of flow through the root to 5 seconds.

For any given root, it is clearly shown that a reduction in temperature makes the apical region more irritable. The amount of current required for

[4] No consistent potential change in any given direction was observed in the series of experiments with dry air; however, an increased positivity did result in some cases.

the demonstration of this effect varies from root to root even when they have been grown side by side in the aquarium. It is not surprising, therefore, that under these conditions roots are found which are unstimulative with 2.00 microamperes even at the relatively low room temperatures at which
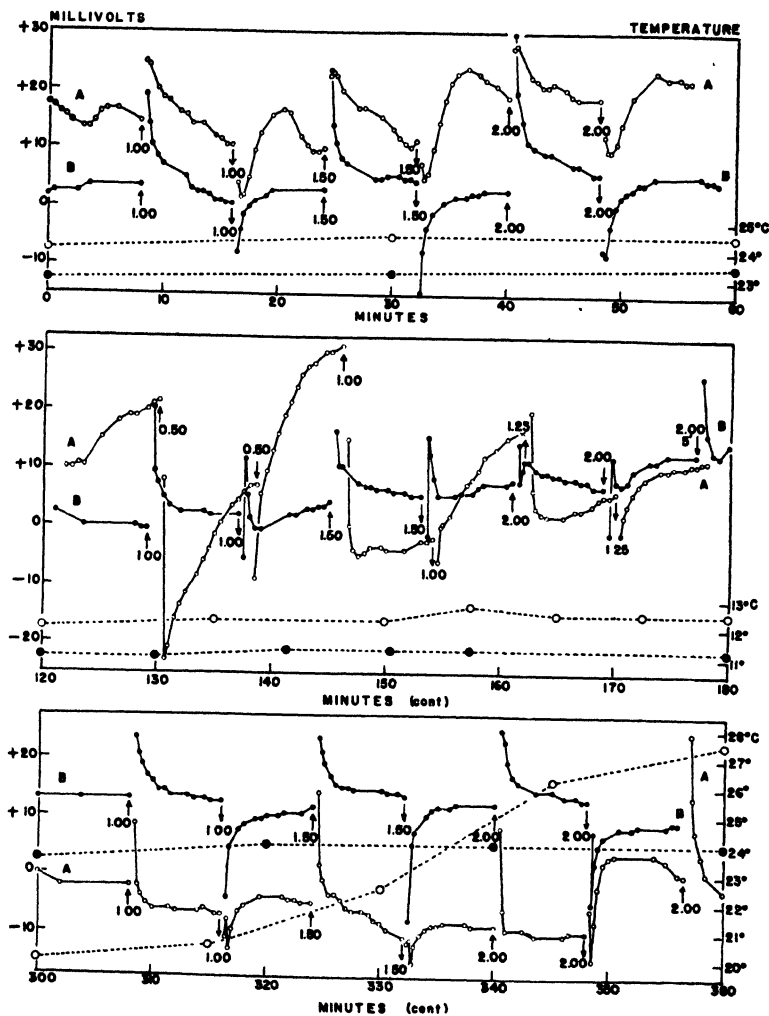
FIG. 7. The effect of temperature on the relative response of apical and basal segments to applied current. The arrows indicate the direction of the applied current. The number with each arrow gives the magnitude of the current in microamperes. Current was applied for 30 seconds and the positions of the contacts were: curve A, 0 and 5 mm.; curve B, 10 and 15 mm. The temperature variation is given by the broken curves. Two separate roots were used for curve A and curve B.

the vast majority of roots are stimulated. In spite of these variations in the behavior of a few roots, it is generally true that larger currents were required for stimulation in summer than in winter. The higher room temperature in Texas coupled with the duration and amount of current employed

probably account for the only occasional observation of stimulation reported in the paper by BERRY (1). Even so, it is difficult to understand why MARSH (11), also working at the University of Texas, failed to find any stimulating effects of currents several times as large as any used in this work. It is not known how much the source and previous history of the onions used for growing roots affects the results but it may well be that some such explanation accounts for the differences.
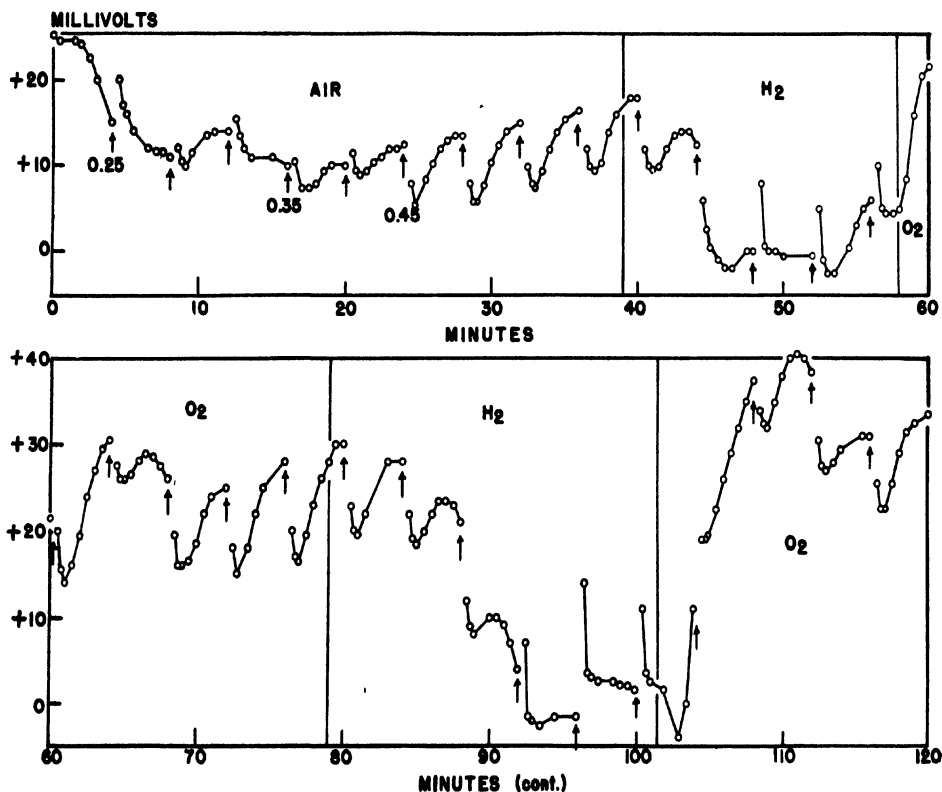
FIG. 8. The effect of hydrogen and oxygen on the response of inherent potential to applied current. The arrows indicate the time at which current was sent up the root. The numbers with the arrows give the magnitude of the current in microamperes. (When no number is given the current was the same as that last indicated.) Current was applied for 30 seconds and the contacts were at 0 and 5 mm. The vertical lines give the time at which the circulation of hydrogen or oxygen was begun.

EFFECT OF HYDROGEN AND OXYGEN ON THE STIMULATING ACTION
OF AN APPLIED CURRENT

The amount of current applied up the root was increased until stimulation occurred and then hydrogen gas was passed through the experimental chamber. A drop in observed potential was produced accompanied by a resultant transition from stimulation to polarization after current flow. Typical of this behavior of roots is the curve presented in figure 8. Contacts were at 0 and 5 millimeters above the tip (inset X, fig. 4). Current

was applied for 30 seconds with a 4-minute interval between applications. The perfect reversibility of this action is clearly demonstrated by the repetition of hydrogen administration. The admission of hydrogen and hence the change from stimulation to polarization required a longer period the second time because oxygen had to be replaced rather than air as originally. The action of these gases in producing the changes in inherent potential described by ROSENE and LUND (18) is nicely illustrated. The large rebound in oxygen after a period in hydrogen is quite prominent the second time oxygen is admitted. The initial drop that precedes this rise is also commonly observed.

Since the removal of oxygen from the root causes the transition from an irritable to a polarizable condition, it was considered of interest to ascertain the effect of a prolonged period without oxygen on the response of the root to applied direct current. Would it be possible to observe a decrease or loss of the polarizability of the root under these conditions? If not, would the reality of the reported difference in the absolute magnitude of polarization and depolarization as well as the difference between these effects in the absence of any stimulation in apical and basal regions become apparent? The answers to these questions are found in the curves in figure 9. These curves are typical of those found with four different roots. Note that the time scale in this figure has been changed in order to confine the results to a single page with each panel two hours rather than one. The current was passed alternately up and then down the root for 15 seconds and eight minutes elapsed between applications. One fifty-minute gap in readings was allowed for recovery and may be found between 280 and 330 minutes.

With the contacts at 0 and 5 millimeters above the root tip as shown in inset X, the usual change from stimulation to polarization is seen soon after the hydrogen started passing through the chamber, curve A. No appreciable alteration in depolarization accompanied this change. The inherent potential fell to a small negative value during the first 50 minutes of this period and then began to rise slowly throughout the remaining 80 minutes, finally reaching a potential of 10 millivolts positive. From 100 to 160 minutes the polarizations were smaller than the depolarizations but both were increasing in size. While hydrogen was still passing through the chamber, the contacts were raised to 10 and 15 millimeters above the tip of the root (inset Y). It is seen that the polarizations were also smaller than depolarizations (curve B) but the difference was not as pronounced as in the apex. Moreover, the magnitude of the basal polarizations and depolarizations were less than those of the apex. When the contacts were returned to 0.0 and 5 millimeters above the tip with the root still in hydrogen there was no further increase in potential over the next 35 minutes and both polarizations and depolarizations reached a constant and steady value (curve A'); the difference in magnitude of the two persists.

Oxygen was circulated through the chamber beginning just before 240 minutes. Instead of the usual "overshooting" phenomenon which is seen
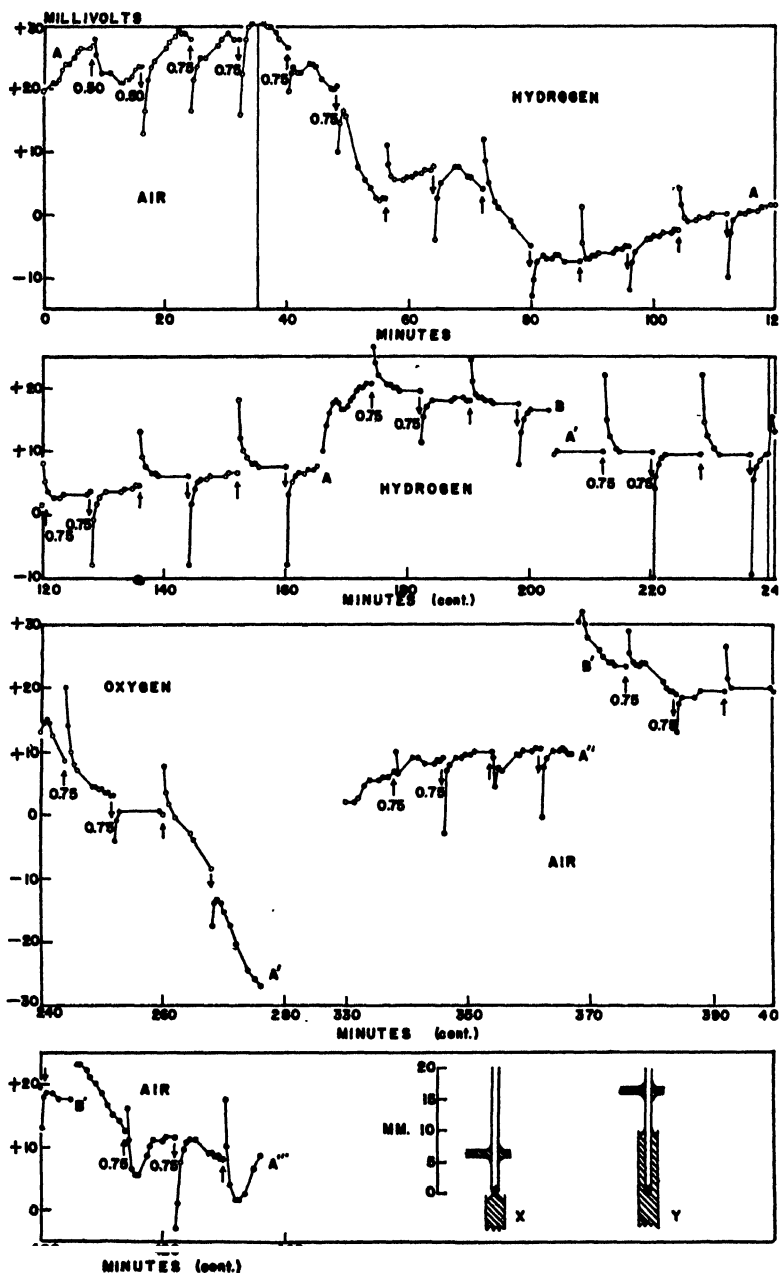
FIG. 9. The effect of hydrogen and oxygen on the relative response of apical and basal segments to applied current. The arrows indicate the direction of the current which was applied for 15 seconds. The numbers give the magnitude of the current in microamperes. (When no number is given the current was the same as that last indicated.) The positions of the contacts were: curves A, 0 and 5 mm. (inset X) and curves B, 10 and 15 mm. (inset Y). The vertical lines give the time at which the circulation of hydrogen or oxygen was begun. All the curves were obtained from the same root.

in figure 8, there was only a small abrupt rise followed by a consistent and appreciable fall in potential that reached nearly 30 millivolts negative over a 35 minute period (curve A'). Moreover, there was no return to stimulation by upward current during this time but the polarizations and depolarizations decreased in size and became more nearly equal. Following the 50-minute recovery period, during which time no readings were made, there was an increase in potential to a positive value as seen in curve A''. The depolarizations were similar to the original ones in air but only a small polarization and a slight stimulation were produced when current passed up the root. The behavior of the basal segment (curve B') was not very different from that in hydrogen except that the changes in potential produced by the current were essentially equal and slightly smaller than before. The contacts were finally returned to the apex and curve A''' was obtained. It is seen that there was no complete recovery of the initial irritability but a definite return in that direction was made.

EFFECT OF AGE ON THE RESPONSE OF THE ROOT TO DIRECT CURRENT

On three successive days the same root was tested with the same amount of direct current passed alternately up and then down the root for 15 seconds with 8 minutes between each application. The root was returned to the aquarium after each experimental period and permitted to grow. The results from two of the five roots so treated are given in figure 10. The contacts were at 0 and 5 mm. above the tip in all cases. The lengths were as follows: the first root for curve A, 30.4 mm.; curve A', 37.6 mm.; curve A'', 50.0 mm.; and the second root for curve B, 30.4 mm.; curve B', 43.8 mm.; and curve B'', 57.3 mm. The diameter of both roots remained unchanged. Remarkably consistent results were obtained in all roots. In every case the only effect of age was a slightly greater change in potential following current flow in the longer, older roots. This took place without any significant difference in the magnitude of the inherent potential. The only root that failed to be definitely stimulated with upward current was the one in curve A when 1.00 microampere was used at 40 minutes. The slight polarization that occurred did not reappear in either A' or A''. Since the magnitude of the response to 0.50 microampere increased slightly in some cases on successive days it might well be that the "threshold" of stimulation was lowered but this was not tested. It seems reasonable to assume on the basis of these results, however, that the range of root lengths used in this investigation yield quite uniform results.

Discussion

Small direct currents when passed through the root are capable of producing two entirely different types of changes in the observed potentials of the system. One type of change would be expected to occur at any electrode at which electric charges can be separated. Hence the existence of polarization or depolarization following current flow would be anticipated on the

basis of well known physical-chemical facts. The oppositely oriented poten-
tial changes following current flow would be expected, however, only as a
result of alterations of membranes or phase boundaries which serve as elec-
trodes within the cell. Such an explanation is the well known and generally
accepted interpretation of muscle and nerve action potentials in biology.
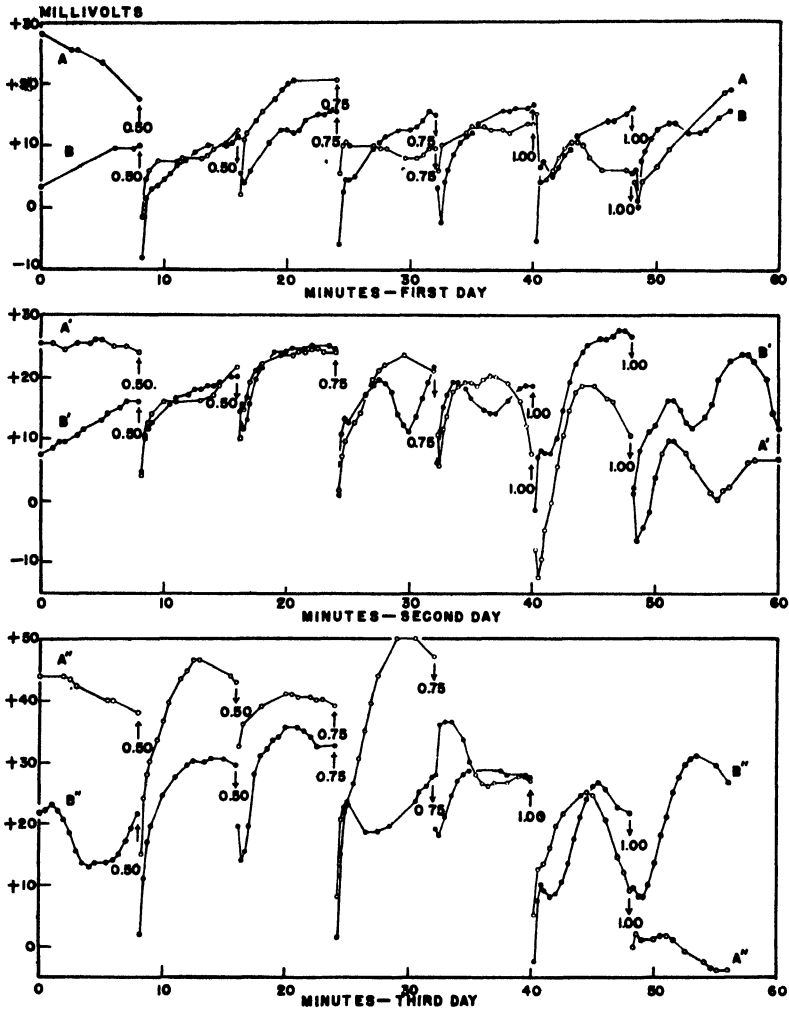
FIG. 10. The effect of root length on the response to applied current. The arrows
indicate the direction of the current, which was applied for 15 seconds. The numbers
give the magnitude of the current in microamperes. The contacts were at 0 and 5 mm.
Curves A, A', and A'' refer to one root on three successive days, and curves B, B', and B''
refer to another root on three successive days.

The recovery process in nerve and muscle following a stimulation is thought
to consist of restoration of the electrode (plasma membrane) across which
the charges are separated. This process has been shown to be dependent on
oxidative metabolism. An examination of our results in the light of this

interpretation makes it apparent that additional considerations are required. It may well be that the difficulties are due at least in part to the complexity of a multicellular root as compared to single muscle or nerve cells. It is also true, however, that strict agreement should not necessarily be expected between plant and animal tissue.

For any root capable of being stimulated, it has been shown that the transition from polarization to stimulation is dependent upon the magnitude of the current. If the electrodes involved were the same for polarization and stimulation this would be anticipated. Similarly, if we were dealing with the same electrical system and the response to applied current depended upon the size of the current, this response would be altered by the condition of the system at the time the current was applied. In other words, a stimulated region or one whose potential was reduced to essentially zero by removal of oxygen should not be polarizable. Under both conditions it is shown that polarization is still possible (figs. 4, 5, 8, 9). Also, the polarization that may follow a longer flow of current than that required for stimulation (fig. 3) is in keeping with the above observations. It is not known, however, whether these observations are due to the presence of two different electrical systems within the root, one of them capable of being stimulated and the other only polarizable under the conditions of these experiments, or whether they are due to the same electrical system which is capable of undergoing rapid and marked changes in irritability depending upon the immediate previous treatment of the root. Cells three millimeters or more above the tip are less easily stimulated than those nearer the apex. In fact, with AC stimulation (3) it has been found that of the first 5 mm. of the tip only the first millimeter will give a potential change and it is as great a change as that given by the entire 5 mm. for a given amount of current. The polarization immediately following a stimulation or that in hydrogen might very well be explained by a decrease in the potential of cells in the region of the basal contact rather than an increase in the potential of the cells near the apical contact. This interpretation is based on the experiments of ROSENE which show that the E.M.F. observed is an algebraic summation of the E.M.F. of all cells between the contacts. Therefore, with the apical cells stimulated it would be necessary either to reduce the potential of the more basal cells or to increase the potential of the apical cells by means of a non-oxidative, non-irritable system in order for the current flow to increase the potential.

It is certainly clear that stimulation requires oxygen while polarization occurs to even a greater extent in its absence (fig. 9). As in muscles and nerves, the restoration of the ''electrodes'' which break down during the passage of current depends upon oxidative processes. The work of LUND (9), and MARSH (13) linking the origin of bioelectric potentials to oxidative metabolism has definitely established that such a dependency exists. The disagreement among the workers in this field concerns the nature of the linkage. This series of experiments requires that any theory must include

some mechanism whereby the intimate dependency between oxygen and stimulation is explained. Similarly, a mechanism that permits polarization and depolarization with or without oxygen and independent of the magnitude or polarity of the inherent potential must be considered. For this last reason, it is thought most likely that within the root it is possible to have both an oxygen sensitive and oxygen insensitive electrical structure or system. The integrity of the electrodes or phase boundaries responsible for polarization and depolarization must be independent of oxygen for several hours at the tension maintained in the experiment. On the other hand, the mechanism of stimulation is inoperative within a short time after the removal of oxygen. According to the discussion above regarding the identity of the two "electrodes" a choice is required between the possibility that all (or most) basal regions have an oxygen insensitive electrical system, which is contrary to the observations of ROSENE and LUND (18) even though they found that the apex was more sensitive, or the possibility that the two systems exist simultaneously. The gradual increase in potential which always occurred with a long period in hydrogen after the initial fall particularly points toward the idea that ionic differences independent of any oxidative processes may be at least partially responsible for applied current effects under these conditions.

Summary

1. The response of the inherent potential of onion roots to applied direct current depends upon the direction and magnitude of the current flow. Weak currents passed up the root cause polarizations but as stronger currents are used the potential may decrease. This effect is reversible. Downward currents usually depolarize the root but may, if sufficiently large and under some conditions, increase the potential. There is a marked variation in individual roots as to the size of current required for the different types of response. In fact some roots only polarize and depolarize without exhibiting the stimulative changes. The apical segments of the root, i.e., those involving the first few millimeters of the tip, are more readily stimulated than more basal segments.

2. Short applications of current produce larger stimulations than longer applications. This effect is largely reversible. If the current is applied for sufficient time a polarization may result. Since a stimulated root may be polarized when current is applied before recovery is complete, it is suggested that the total response of the inherent potential to current flow may be an apparent algebraic summation of a stimulation and polarization with the latter increasing as time of flow increases.

3. Temperature of the environment is an important factor in determining the nature of the response of a root to current flow. Lower temperatures render a root more easily stimulated, especially the apical segment. There is considerable variation in the behavior of individual roots in this respect, however.

4. A root which is stimulated in air by current flow can only be polarized in hydrogen. This is perfectly reversible as oxygen replaces the hydrogen and may be repeated several times with consistent results. In the absence of any detectable stimulating effect of current in an atmosphere of hydrogen, it is found that the magnitude of polarizations is less than depolarizations in any given root segment and that both are larger in apical segments than the corresponding change in basal segments.

5. No appreciable change in the response of inherent potential to current flow is produced by growth of the root between 30 and 55 mm. and thus the individual variation of roots may not be attributed to this factor.

6. It is suggested that polarization and depolarization resulting from current flow are due to the accumulation of free ions at "electrodes" within the root. These "electrodes" are not dependent upon oxygen for their integrity nor are they affected by stimulation. On the other hand stimulation must depend upon the breakdown of oxygen-sensitive "electrodes."

It is with pleasure that we acknowledge the technical assistance of Miss ATHLEEN R. JACOBS during part of this investigation.

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LITERATURE CITED

1. BERRY, L. JOE. Polarization potentials of short duration and their independence of the inherent polarity potential in the onion root. In press.

2. ————. Spontaneous variations in the electrical potentials of the root of *Allium cepa*. Thesis, University of Texas. 1939.

3. ————, and HOYT, ROSALIE C. Stimulation of the onion root by alternating current. Unpublished data.

4. BLINKS, L. R. The effect of current flow on bioelectric potential. I. Valonia. Jour. Gen. Physiol. **19**: 633–672. 1935.

5. ————. The effect of current flow on bioelectric potential. II. Halicystis. Jour. Gen. Physiol. **19**: 867–898. 1935.

6. ————. The effect of current flow on bioelectric potential. III. Nitella. Jour. Gen. Physiol. **20**: 229–265. 1936.

7. ————. The relations of temperature to the potassium effect and the bioelectric potential of Valonia. Jour. Gen. Physiol. **25**: 905–916. 1942.

8. CLARK, W. G. Electrical polarity and auxin transport. Plant Physiol. **13**: 529–552. 1938.

9. LUND, E. J. Relation between continuous bioelectric currents and cell respiration. II. Theory of cell correlation. Jour. Exp. Zool. **51**: 265–290. 1928.

10. ————, and MOORMAN, J. B. Electrical polarity and velocity of cell oxidation as functions of temperature. Jour. Exp. Zool. **60**: 249–264. 1931.

11. MARSH, G.   The effect of applied electric currents on inherent cellular
    EMF and its possible significance in cell correlation.   Proto-
    plasma 11: 447–474.   1930.

12. ——————.   The effect of mechanical stimulation on the inherent
    EMF of polar tissues.   Protoplasma 11: 497–520.   1930.

13. ——————.   Kinetics of an intracellular system for respiration and
    bioelectric potential at flux equilibrium.   Plant Physiol. 10: 681–
    697.   1935.

14. ——————.   The effect of temperature on the inherent potential of
    Valonia.   Carnegie Inst. Washington.   Pub. no. 475.   1936.

15. REHM, W. S.   Electrical response of *Phaseolus multiflorus* to electrical
    currents.   Plant Physiol. 14: 359–363.   1939.

16. ROSENE, H. F.   Proof of the principle of summation of cell E.M.F.'s.
    Plant Physiol. 10: 209–224.   1935.

17. ——————.   The effect of an applied electric current on the external
    longitudinal polarity potentials of Douglas fir.   Amer. Jour. Bot.
    24: 390–399.   1937.

18. ——————, and LUND, E. J.   Linkage between output of electric
    energy by polar tissues and cell oxidation.   Plant Physiol. 10:
    27–47.   1935.

19. WILKS, S. S.   The electric potentials in the coleoptile of *Avena sativa*.
    Thesis, University of Texas.   1936.

PREPARATION OF SYNTHETIC COMPOSTS FOR MUSHROOM CULTURE[1]

B. B. STOLLER

(WITH TWO FIGURES)

Introduction

Horse manure and its straw bedding is the standard medium in commercial mushroom culture today. Since horse manure has become scarce and increasingly costly in our automobile age, attempts have been made to find substitutes during the past twelve years. In order to produce an economical and competitive "artificial manure" or "synthetic compost" it is necessary to use other wastes, especially industrial and agricultural byproducts. The method of preparing synthetic composts described in this paper has been tested over a period of five years; several thousand tons have been prepared, and the yield has been as good as or better than that from horse manure composts.

The history of "artificial manures" began in 1923 when RICHARDS and HUTCHINSON (5) patented a method for preparing "nitrogen fertilizers" from straw and inorganic nitrogen. In a later patent (6) phosphates also were included. The gist of their method is that 3.6 lb. of nitrogen, preferably in a soluble form, should be added to 500 lb. of straw; larger quantities of nitrogen produced a high alkalinity which checked the decomposition of the straw and caused a loss of nitrogen. In 1930, LAMBERT (3) and HEIN (2) attempted to use this method for preparing a compost for mushroom culture. The yield of mushrooms they obtained from such composts was less than one-half of the yield from horse manure composts. Their investigation demonstrated that normal mushrooms could be produced from an artificial manure even though the yield was unsatisfactory.

Later WAKSMAN and RENEGER (15) prepared synthetic composts by adding alfalfa and ammonium phosphate to straw. Their aim in this use of alfalfa was to facilitate the decomposition of the straw by adding to it a "green material" with a high moisture content. The yield of this compost was 70 per cent. of that of a manure compost. They also prepared composts of tobacco stems and straw; the yields of these were variable.

Recently SINDEN (7) prepared synthetic composts consisting of wheat straw, urea, and wheat. Urea was selected as a source of nitrogen in preference to other compounds because it left "no inorganic residue." The wheat was added to help retain the heating capacity of the compost. The best yields he obtained with such composts were about two-thirds of the yields with manure composts, per square foot of bed surface.

This brief review of the attempts to prepare synthetic composts for mushroom culture shows that only nitrogen has been considered significant, so

[1] This paper is a contribution of the L. F. Lambert Research Laboratory, Coatesville, Pennsylvania.

that the composts prepared by these investigators are essentially the same as the "nitrogen fertilizer" of Richards and Hutchinson. Comparatively slight attention has been given to the need for phosphates and no attempt has been made to observe the necessity for potassium salts. The composts of these investigators refer only to specific mixtures; no means are provided whereby materials other than those specifically mentioned might be substituted. Thus it would be hazardous to deviate from any specific combination since the function of each constituent used is not fully understood. While these investigators have increased our knowledge of synthetic composts, they have not developed a compost which will consistently produce a yield of mushrooms equal to or greater than that produced by a compost of horse manure and its straw bedding.

⌡ Basis of present method

The basis for the method of preparing synthetic composts in the present investigation was to analyze both the medium (horse manure) and the product (the mushroom) for their chief chemical constituents, and then seek

TABLE I

AN AVERAGE ANALYSIS OF HORSE MANURE AND ITS STRAW BEDDING

MINERAL CONSTITUENTS	PERCENTAGE, DRY BASIS	AMOUNT IN ONE TON (70% MOISTURE)
	%	lb.
N	2.0	12.0
P_2O_5	1.0	6.0
K_2O	2.7	16.0

to produce synthetically a medium which would contain these significant constituents. The composition of horse manure is variable. When numerous samples have been analyzed, however, and when the analyses are compared with those of other investigators, a workable average may be obtained as shown in table I. The analysis of the ash and organic matter of the mushroom is shown in table II. These analyses show that nitrogen, phosphorus, and potassium are present in significant quantities in both the medium and the product, and must all be used in preparing synthetic composts.

As a result of extensive experimentation, it has been found that a ton of synthetic compost with 70 per cent. moisture should contain about 13 lb. N, 4 lb. P_2O_5, and 10 lb. K_2O. It would seem, then, that manure contains more P_2O_5 and K_2O but less N than is necessary to produce the largest yield of mushrooms. The writer believes the reason for artificial manure, in which only the nitrogen factor has been given due consideration, yielding normally "when mixed half and half with composted horse manure" (4), is because superfluous quantities of P_2O_5 and K_2O are present in the manure. The ratio of N to P_2O_5 to K_2O found in the mushrooms (6.4:2.4:4.4) appears to be about the same as has been found satisfactory for preparing synthetic composts. This analysis of the mushroom may, however, reflect

TABLE II

AN ANALYSIS OF THE ASH AND ORGANIC MATTER OF THE MUSHROOM (SPOROPHORE),
Agaricus campestris

MINERAL CONSTITUENTS OF ASH (1)	PERCENTAGE	ORGANIC CONSTITUENTS OF DRY MATERIAL (13)	PERCENTAGE
	%		%
K_2O	43.94	Ether soluble portion	5.14
Na_2O	2.31	Hot water-soluble organic matter	42.16
CaO	1.32	Alcohol soluble portion	7.02
MgO	0.21	Hemicelluloses	13.66
Fe_2O_3	0.24	Cellulose	4.86
Mn_2O_3	0.02	Lignin	0.92
Al_2O_3	2.31	Protein	40.35
P_2O_5	24.25	Nitrogen	6.44
SiO_2	8.23		
Cl	9.22		
SO_3	3.01		

only the specific condition of these constituents in the particular manure on which the mushrooms grew.

The procedure in accordance with the method (10) herein described is to analyze the materials to be used in preparing the synthetic composts into their N, P_2O_5, and K_2O constituents as shown in table III. The straw and root composts are equivalent to a ton of manure with 70 per cent. moisture. In the computation of these composts, the N, P_2O_5, and K_2O in the fibrous material is considered first; any deficiencies of these constituents are then supplied from various sources. The number of sources is immaterial as long

TABLE III

TABULATION OF THE CONSTITUENTS OF SYNTHETIC COMPOSTS

MATERIALS	QUANTITY	N	P_2O_5	K_2O
LICORICE ROOT COMPOST				
	lb.	lb.	lb.	lb.
Spent licorice roots (67.5% moisture)	2000.0	6.5	1.0	
Dried brewers' grains	180.0	7.4	1.8	
Sulphate of potash (50% K_2O)	18.0			9.0
Muriate of potash (50% K_2O)	3.0			1.5
Superphosphate (20% P_2O_5)	7.5		1.5	
Hydrated lime [94% $Ca(OH)_2$]	6.0			
Totals		13.9	4.3	10.5
STRAW COMPOST				
Rye straw	500.0	2.5	1.0	5.0
Wet brewers' grains	660.0	7.0	1.6	
Uramon (42% N as urea)	8.3	3.5		
Superphosphate (20% P_2O_5)	10.0		2.0	
Sulphate of potash (50% K_2O)	9.0			4.5
Muriate of potash (50% K_2O)	3.0			1.5
Hydrated lime [94% $Ca(OH)_2$]	9.0			
Totals		13.0	4.6	11.0

TABLE IV

THE PREPARATION OF SYNTHETIC COMPOSTS WITH SPENT LICORICE ROOTS AND VARIOUS
SOURCES OF NITROGEN. THE RATIO OF $N:P_2O_5:K_2O$ IS ON THE AVERAGE: $15:5:10$.
SUPERPHOSPHATE (20% P_2O_5) AND SULPHATE OR MURIATE OF POTASH (50%
K_2O) ARE USED TO SUPPLEMENT ANY DEFICIENCIES OF PHOSPHORUS
AND POTASSIUM IN THE ROOTS AND SOURCES OF NITRO-
GEN TO ATTAIN THIS RATIO

PLOT	SOURCES OF NITROGEN	ADDED PER TON OF ROOTS	NITRO GEN PER TON OF ROOTS	SIZE OF PLOT	MUSH-ROOMS PER PLOT	MUSH-ROOMS PER SQ. FT.	COM MERCIAL YIELD* PER SQ. FT.
		lb.	lb.	sq. ft.	lb.	lb.	lb.
38	Dried blood	60.0	7.8	48	111	2.31	1.73
40	Cottonseed meal	120.0	8.0	48	113	2.35	1.77
41	Soybean meal	123.0	8.0	48	141	2.94	2.20
42	Castor bean meal	145.5	8.0	48	129	2.69	2.02
43	Malt sprouts	189.5	8.0	48	164	3.42	2.56
46	Beet molasses	200.0	2.8	48	92	1.92	1.44
	Ammophos	20.0	3.2				
	Urea	4.4	2.0				
47	Tobacco stems (ground)	150.0	3.8	48	97	2.02	1.52
	Urea	10.0	4.5				
48	Cottonseed meal	40.0	2.7	48	129	2.69	2.02
	Dried blood	20.0	2.6				
	Urea	5.5	2.7				
49	Dried blood	7.7	1.0	24	64	2.67	2.00
	Cottonseed meal	15.0	1.0				
	Soybean meal	15.4	1.0				
	Dried brewers' grains	23.4	1.0				
	Dried yeast	2.5	0.2				
	Urea	8.6	4.0				
49A	Same as 49			24	61	2.54	1.91
51	Dried tannery sludge	200.0	4.0	48	119	2.48	1.86
	Urea	8.6	4.0				
52	Same as 51 plus ''Ultra Life'' —(Vitamin feed conc.)	10.0		48	131	2.73	2.05
53	''Uramon'' (urea with 42% N)	20.4	8.5	24	27	1.13	0.84
53A	Same as 53 plus 2 lb. more lime			24	47	1.96	1.47
54	''Uramon''	22.8	9.5	48	143	2.98	2.24
	Chopped straw	75.0					
	Cheese whey	10.0					
55	Calcium nitrate	63.5	9.5	48	136	2.83	2.13
58	Ammonium sulphate	42.5	8.5	48	None		
59	Ammonium sulphate	47.5	9.5	24	59	2.46	1.84
	Chopped corn cobs	100.0					
	Cheese whey	10.0					
59A	Same as 59 plus 2 lb. more lime			24	39	1.63	1.22
60	Dried brewers' grains	200.0	8.2	48	151	3.15	2.36
66	Same as 60 plus different source of potash	200.0	8.2	48	148	3.08	2.31
70	Dried brewers' grains	100.0	4.1	24	71	2.96	2.22
	Urea	9.2	4.2				
75	Dried brewers' grains	100.0	4.1	48	157	3.27	2.45
	Dried blood	7.7	1.0				
	Cottonseed meal	15.0	1.0				
	Soybean meal	15.4	1.0				
	''Uramon''	4.8	2.0				

TABLE IV—(*Continued*)

PLOT	SOURCES OF NITROGEN	ADDED PER TON OF ROOTS	NITRO-GEN PER TON OF ROOTS	SIZE OF PLOT	MUSH-ROOMS PER PLOT	MUSH-ROOMS PER SQ. FT.	COM-MERCIAL YIELD* PER SQ. FT.
76	Same as 75 plus 6 lb. more lime			48	163	3.40	2.55
77	Same as 75 plus 6 lb. more of magnesium lime			48	139	2.90	2.17
78	Extracted cocoa cake	200.0	6.0	48	147	3.06	2.30
	Urea	7.6	3.5				
79	Extracted cocoa cake	100.0	3.0	48	129	2.69	2.02
	Urea	13.0	6.0				

* Commercial yield: Since the mushrooms, as weighed for the cannery, included the stubs, a reduction of 25% is allowed, so as to conform with the weight of mushrooms cut for the market. The average weight of the stubs, with adhering soil, is 22% of the weight of the mushroom.

as they add up to the total quantities and ratio desired. Synthetic composts prepared from many different sources of N, P_2O_5, and K_2O gave equally good yields. This fact shows that these three constituents are available for the most part to the mushroom mycelium in the various sources investigated. The writer has observed large particles (of chemicals), which were very insoluble in water or in agar medium, oxidized by the growing mycelium.

In preparing the compost pile, the nitrogenous material is spread or sprayed over the surface of the widened heap of fibrous material and forked in about a foot. All mineral ingredients are then mixed together, diluted with an equal quantity of loam, and spread evenly over the top of the heap. The whole mass is then mixed, watered as it is turned, and arranged into a pile which will provide the necessary aeration.

This method of preparing composts by analyzing and tabulating the various materials affords a formulation in which various agricultural and industrial by-products may be substituted. In order to compete with a waste product like manure, the possibility for substitution is important; it is necessary to have a formula in which the least expensive by-products on the market may be used. This method also establishes a basis for the estimation of the quantity of a material required in a synthetic compost.

Materials for synthetic composts

Since the mushroom is a fungus, an organic source of carbon has to be supplied. Straw which has undergone a microbial decomposition is a satisfactory source. The straw also provides for the mushroom bed the fibrous structure which is necessary for the intensely aerobic growth of the mushroom mycelium. Fibrous materials like spent licorice roots (from which the licorice has been extracted) and spent tannery nuts, bark, and leaves (from which tannin has been extracted for industrial use) are even more satisfactory than straw. These materials already contain sufficient moisture, are suitably subdivided, and require only a short or no microbial decomposition.

The use of numerous nitrogen sources for synthetic composts is shown in table IV. Composts with some nitrogen sources gave better yields than with others. The yields, however, were affected by the composting period which, at 30 days, was rather long for these small plots; in a later consideration of the problem of composting it seemed that the yields of some of the plots would have been better if the composting period had been shorter.

Organic nitrogenous materials are preferable to inorganic because they serve also as a source of carbon, contain some K_2O and P_2O_5, and have a better heating capacity. A solution of inorganic nitrogenous substances absorbed in very finely ground straw or corn cobs, however, is a fair equivalent to the organic material. The selection of a suitable nitrogen source also depends, of course, on the cost of the material compared to the yield of mushrooms obtained by its use. The quality of the mushrooms produced by the use of any specific material is not superficially evident; possible intrinsic values are not commercially important at present.

Some organic nitrogenous materials may be unsatisfactory due to the presence of specific toxic constituents as in the case of cocoa shells and cake. These materials contain one per cent. or more theobromine which, at this concentration, prevents the growth of the mushroom mycelium. When the theobromine is extracted, however, as is the practice in the commercial manufacture of theobromine, the resulting extracted cocoa cake is a satisfactory source of nitrogen.

In the use of inorganic nitrogenous substances like ammonium salts, cyanamide, and urea special precautions are necessary; but nitrates may be used freely. Ammonia formed from the former substances persists in the compost (due to adsorption) even after the "sweating out process" and prevents the growth of the mushroom mycelium. One of the simplest methods for suppressing this excessive ammonification is to absorb solutions of the ammoniating substances in finely ground carbonaceous materials as already indicated; in this way the transformation of ammonia to microbial proteins is assisted; the use of an acidifying material like cheese whey is also helpful. If calcium cyanamide is improperly stored, that is, exposed to air so that it absorbs moisture and carbon dioxide, it is transformed to calcium dicyanodiamide. Composts prepared with dicyanodiamide even prevent the growth of the mushroom mycelium. In contrast to ammoniating salts, nitrates are not toxic to the mycelium. Nitrates are especially suitable for use with fibrous materials which do not undergo extensive microbial decomposition. Composts of nitrates with spent licorice roots or spent tannery bark require no outdoor composting.

The variety of sources of the other materials employed in the preparation of synthetic composts is comparatively limited. The best source of phosphates is superphosphate. Besides the fertilizer grades of potassium chloride and sulphate, the ash of various plant materials such as cottonseed hulls, and the ash of the sludge of alcohol distilleries may also be used. Current investigations, which will be reported at a future date, indicate that the

"minor elements" are related to disease control.   The addition to composts of yeast and vitamin concentrates failed to significantly increase the yield; the vitamin content of the mushroom, however, may have been increased.

TABLE V

THE EFFECT OF POTASH, PHOSPHATE, AND LIME ON THE YIELD OF MUSHROOMS WHEN ADDED TO A SYNTHETIC COMPOST CONSISTING OF SPENT LICORICE ROOTS · AND BREWERS' GRAINS

PLOT	FERTILIZER SALTS PER TON OF COMPOST				MUSH-ROOMS PER 24 SQ. FT.	MUSH-ROOMS PER SQ. FT.	AVERAGE
	K_2SO_4, 50% K_2O	KCl, 50% K_2O	SUPER-PHOS-PHATE, 20% P_2O_5	HYDRATED LIME, 94% Ca(OH)$_2$			
	lb.	lb.	lb.	lb.	lb.	lb.	lb.
61	18	3	5	None	65	2.71	2.6
81					58	2.42	
62	18	3	None	6	52	2.16	2.1
82					50	2.10	
63	None	None	5	6	38	1.60	1.4
83					27	1.13	
64	18	3	5	6	55	2.30	2.4
84					59	2.46	
65	18	3	5	3	60	2.50	2.6
85					62	2.60	
66	18	3	5	9	58	2.42	2.4
86					57	2.38	
67	18	3	2.5	6	74	3.10	2.7
87					56	2.34	
68	18	3	7.5	6	50	2.10	2.3
88					59	2.46	
69	12	2	5	6	48	2.00	2.1
89					54	2.25	
70	24	4	5	6	49	2.04	2.0
90					49	2.04	
71	None	None	None	None	32	1.33	1.1
91					21	0.88	
72	"	"	"	6	21	0.88	0.8
92					16	0.67	
73	"	"	5	None	31	1.30	1.4
93					35	1.46	
74	18	3	None	"	50	2.10	2.1
94					52	2.16	
75	None	None	"	"	22	0.92	0.9
95					23	0.96	
Manure C	"	"	"	"	52	2.16	
" D	"	"	"	"	50	2.10	2.3
" E	"	"	"	"	60	2.50	
" F	"	"	"	"	56	2.34	

The effect of potash

Synthetic composts of spent licorice roots as shown in table III afford an excellent opportunity to test the effect of potash on the yield of mushrooms. Practically all of the potash has been washed out of the licorice roots during the hot water extraction; similarly, most of the potash is removed from the brewers' grains during the extraction of the malt.   Furthermore, the spent

licorice roots are ideal for plot experiments; the roots have been cut into ·1- to 2-inch lengths and vary from fine threads to fibers ⅜ inch in diameter. The roots retain moisture evenly and handle easily. An eight-ton mixture of the roots and brewers' grains was prepared and composted for a month. At the end of this time the pile was divided into 16 heaps; potash, superphosphate, and lime were added to these heaps; they were then thoroughly mixed and split into two plots as shown in table V. The duplicate plots were placed on different shelves in the mushroom house.

The results of the effect of potash are shown in table V. Due to over-composting and cooling of the compost when making up the plots, the mushroom house did not heat properly during the sweating out process. As a consequence, insect trouble was encountered and the yields were not altogether satisfactory. The effect of the addition of potash, however, is clear: by its use the yield was almost double that of plots to which only N and P_2O_5 were added. Other experiments with plots containing a ton of compost substantiate this conclusion. The effect of adding phosphates is not distinct because some three-fourths of the phosphates required were already present. Lime had no effect on the yield under the conditions of this experiment.✓

Composting

After all the necessary ingredients are supplied the next important step is the fermentation or "composting" of the materials. The process of composting consists in the microbial decomposition of the carbonaceous materials, in the synthesis of microbial proteins, and in the conditioning of the fibrous materials to absorb and retain moisture. WAKSMAN and NISSEN (14) have shown that as a result of the composting of manure there is a reduction in water-soluble substances, hemicelluloses, and to less extent in cellulose. This is accompanied by an increase in lignin, total nitrogen (which is present chiefly in the form of insoluble microbial proteins), and ash. The experiments of WAKSMAN and IYER (12) indicate that lignin and microbial proteins form complexes. It appears that the nitrogen of these ligno-protein complexes is unavailable unless the lignin is oxidized. It is assumed that the mushroom mycelium can oxidize lignin because the lignin content of the compost decreases as a result of the growth of the mycelium. The writer (11) has observed that the mycelium forms highly colored products when lignin, tannin, and many aromatic hydroxy and amino compounds are added to an agar medium. These colored products appear to be the result of oxidations by the mycelium. The mushroom mycelium has strong oxidizing powers which enable it to utilize the insoluble nitrogen of the ligno-protein complexes by oxidizing the relatively toxic lignin. Since the mycelium has a nitrogen source at its disposal which is unavailable to most of the microorganisms present in the compost heap, it is able to consume the carbonaceous materials not used previously.

The length of the composting period is of paramount importance, although the determination of the length is still an art. The experienced

grower considers the composting sufficient when the manure has a dark color (indicating the liberation of lignins by the consumption of other constituents) and when the tensile strength of the straw in the manure is low (indicating that the straw can absorb and retain water). From the point of view of preparing synthetic composts the length of the composting period is influenced by the nature and subdivision of the fibrous material, by aeration of the compost pile, and by the use of lignin and tannin extracts. These factors will now be considered.

When the fibrous material is the same as that present in manure composts, i.e., straw, then the composting period is relatively long—about 4 to 5

TABLE VI

THE EFFECT OF CHOPPING THE STRAW OF SYNTHETIC STRAW COMPOSTS ON THE YIELD OF MUSHROOMS (AS SHOWN IN TABLE III)

PLOT	LONG STRAW	CHOPPED* STRAW	BED SURFACE	MUSH-ROOMS PER PLOT	MUSH-ROOMS PER SQ FT.	COMMERCIAL YIELD PER SQ. FT.
	lb.	lb.	sq. ft.	lb.	lb.	lb.
1	2000	2000	696	2484	3.57	2.7
2	2000	None	384	1273	3.32	2.5
4	250	250	48	142	2.96	2.2
4A			48	142	2.96	
5	None	500	48	98	2.04	1.7
5A			48	115	2.40	
12–2	200	250	36	96	2.67	2.1†
12–2A			45	132	2.93	
12–3	None	450	39	111	2.85	2.1†
12–3A			36	103	2.84	
12–4	450	None	50	101	2.00	1.8†
12–4A			50	138	2.76	
Check	Manure		3600	12003	3.33	2.5

* Straw was chopped with a hammer mill, using a one inch screen.
† These results are typical of 4 or more replications in which different nitrogen sources were used.

weeks. It is necessary to cut the straw in order to facilitate the retention of heat and moisture both during the composting period and during the "sweating out process" (8). Cut straw is also easier to handle; long straw binds and is laborious to turn. By subdivision of the straw the length of the composting period may be reduced. Before adding the various ingredients to the straw, its moisture content is conveniently brought up to about 50 per cent. by placing a revolving lawn sprinkler on top of the straw pile, which should be about 6 ft. high. It is sprinkled until water leaches from the pile; the watering is continued for several days. Rye and wheat straw are preferable to oat straw or corn stover since the latter have a tendency to lose their fibrous structure when the microbial decomposition has proceeded to the stage where moisture is retained well.

The effect of chopping the straw on the yield of mushrooms is shown in table VI. The results show that chopping the straw caused a reduction in the number of square feet of bed surface and also a reduction in the yield for a given quantity of straw. The reason for the plots with the entire straw chopped giving less mushrooms per plot is because all the plots were composted for an equal length of time; the plots with all chopped straw composted faster than the plots in which the straw was partially or not chopped. When the chopped straw was arranged in plots with the *same* bed surface area as the partially chopped straw (plots 4 and 5), the yield per sq. ft. of bed surface was greatly reduced. If allowance was made for the shrinkage due to the rapidity of composting (all the beds made up to a six-inch depth regardless of the resulting surface area) and if the composting period was shorter as in plots 12–2,3,4, then there is no marked difference in yield.

TABLE VII

THE EFFECT OF THE LENGTH OF COMPOSTING AND THE VENTILATION OF LICORICE ROOT
COMPOST ON THE YIELD OF MUSHROOMS

PLOT	DATE OF ARRANGE- MENT OF PLOT, 1938	NUMBER OF DAYS COM- POSTED	NUMBER OF TIMES COMPOST WAS TURNED	COMPOST IN PLOT WHEN ARRANGED	BED SUR- FACE	MUSH- ROOMS PER PLOT	MUSH- ROOMS PER SQ. FT.	COM- MERCIAL YIELD PER SQ. FT.
				lb.	*sq. ft.*	*lb.*	*lb.*	*lb.*
B–1	Aug. 18	16	3	950	48	167	3.48	2.60
B–2					48	163	3.40	2.54
B–5	Aug. 25	8	2	950	48	187	3.90	2.92
B–6					48	179	3.73	2.79
B–7	Sept. 1	2	1	950	48	115	2.40	1.79
B–8					48	80	1.67	1.25
B	Aug. 17	17	3	25000*	1416	2871	2.03	1.52
2†	Oct. 26	21	3	22000*	1152	4336	3.76	2.82

* Approximately. Each of these piles is typical of 3 others prepared at the same time.
† Ventilator placed in pile at the second turning.

Even though a larger yield for a given quantity of material is obtained from composts of long straw, the best commercial practice is to cut the straw in view of the disadvantages of using long straw.

The composting period is comparatively brief when the fibrous materials are spent licorice roots or spent tannery bark, nuts, and leaves. The reason for these materials requiring less composting is that their chemical composition is more or less similar to composted manure; *i.e.*, a large percentage of lignin or tannin and cellulose, and a small amount of easily decomposable carbonaceous matter. These materials contain about 65 per cent. moisture when purchased, and have other desirable qualities already described. They make better composts than straw.

Synthetic composts prepared with spent licorice roots and composted for 15 days in 1937 gave commercial yields of 2.5 lb. per sq. ft. of bed surface, when prepared in plots of a ton or less. As a result, large piles of licorice

root compost, as plot B in table VII, were prepared the next year. The yields of the large piles were unsatisfactory; however, small plots (B1 to B8, table VII) arranged at the same time to determine the length of the composting period gave good yields. It was evident, then, that the composition of the synthetic compost was satisfactory, but that the method of composting was improper. The small piles had a relatively large surface exposure and only a small "core" removed from the atmosphere, so that almost all parts of the small piles received good aeration; the reverse was the condition present in the large piles. By placing a ventilator, made of a lattice-work of
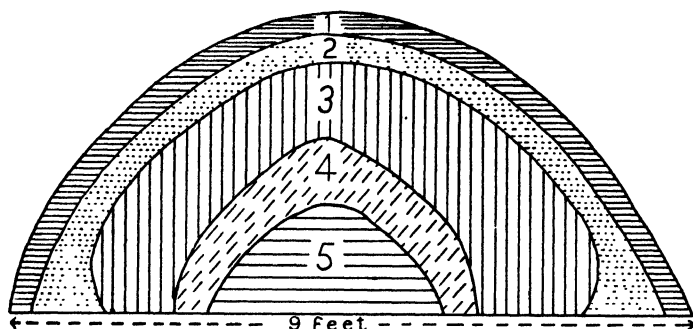
FIG. 1. Cross section of synthetic licorice root compost pile without ventilator. Measurements were made after composting 12 days, at the second turning.

ZONES	MOISTURE	pH	TEMPER-ATURE	SIDE OF PILE		TOP OF PILE
				CO_2	O_2	CO_2
	%		°F.	%	%	%
1. Wet-ammonia	75	8.2	120	2.5	17.4	8.0
2. Fire fang	63	7.9	140	7.8	12.1	11.0
3. Dark brown	64	8.1	165	14.1	5.8	15.5
4. Sour-green	64	5.8	160	24.3	0.2	22.0
5. Very sour-green	66	5.2	140	24.9	1.6	22.2

wood, in the center of the pile good aeration was also obtained in the large piles. The yields from the aerated piles was satisfactory as shown in plot 2, table VII. The conditions of moisture, pH, temperature, and aeration before and after placing a ventilator in the large piles is shown in figures 1 and 2. When preparing composts with straw the problem is to compress the straw as much as possible in order to prevent too much aeration from drying out and cooling the straw compost, whereas with closely packed materials like licorice roots it is necessary to make special provisions for aeration. STOLLER, SMITH, and BROWN (9) demonstrated that manure could be composted in about 7 days when good aeration was provided.

The results in table VII show that the best yields are obtained with small piles of licorice root composts by composting for 8 days. Composting twice as long reduces the yields somewhat; composting only 2 days reduces the

yields drastically and encourages all kinds of mold growth. The large quantity of nitrogen used in preparing these synthetic composts necessitates a composting period of some 8 days. While 8 days of composting suffices in small piles, about 15 to 20 days are required in large piles with ventilators. It is not as simple to obtain proper mixing, watering, and aeration in large piles as it is in small ones.

Since one of the chief functions of composting is to make the nitrogen unavailable to most microorganisms by its combination with lignin, the possibility occurred of achieving this combination without the lengthy microbial decomposition. Accordingly, several nitrogen sources as shown in table
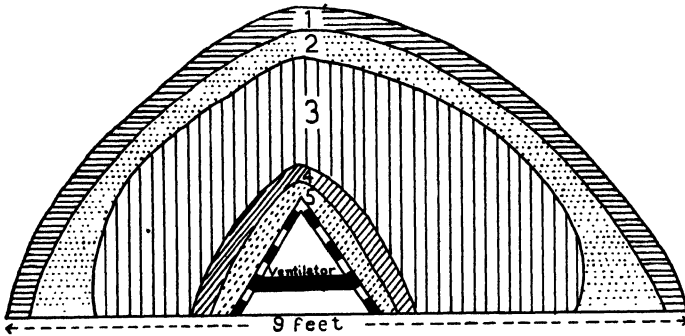
FIG. 2. Cross section of synthetic licorice root compost pile with ventilator. Measurements were made after composting 12 days, at the second turning.

ZONES	MOISTURE	pH	TEMPER-ATURE	SIDE OF PILE		TOP OF PILE
				CO_2	O_2	CO_2
	%		°F.	%	%	%
1. Wet-ammonia ..	75	8.2	120	3.5	17.5	4.5
2. Fire fang	58	7.7	140	12.0	8.3	4.0
3. Dark brown	66	8.0	160	9.0	11.1	3.0
4. Dry	66	7.7		4.5	15.7	1.8
5. Fire fang	63	7.9	140	2.4	18.4	0.7

VIII were soaked in solutions of lignin extracts of the paper industry and in solutions of tannin extracts. Exact details of the procedure are described elsewhere (10). Substances like lignin or tannin are called "coprinating" agents.

The results in table VIII show that when concentrated nitrogenous materials are soaked in solutions of lignin or tannin extracts, the outdoor composting process is unnecessary. In most cases the yields were better without than with composting when lignin or tannin was used. Some nitrogenous materials gave better yields with tannin than with lignin, and vice versa. It is interesting to note that the plots with "Uramon" (a Dupont product in which the urea is diluted to 42 per cent. N) gave a good yield of mushrooms without composting when combined with lignin and tannin.

TABLE VIII

SYNTHETIC LICORICE ROOT COMPOSTS PREPARED BY THE COMBINATION OF TANNIN OR LIGNIN WITH NITROGENOUS MATERIALS.
EACH PLOT HAS A BED SURFACE OF 48 SQUARE FEET

NITROGEN SOURCES	NITROGEN PER TON OF LICORICE ROOTS	"COPRINATING" AGENT	AGENT PER TON OF LICORICE ROOTS	PLOTS COMPOSTED 30 DAYS OUT-OF-DOORS			PLOTS COMPOSTED 7 DAYS OUT-OF-DOORS			PLOTS NOT COMPOSTED OUT-OF-DOORS			
				PLOT	MUSH-ROOMS PER PLOT	MUSH-ROOMS PER SQ. FT.	PLOT	MUSH-ROOMS PER PLOT	MUSH-ROOMS PER SQ. FT.	PLOT	MUSH-ROOMS PER PLOT	MUSH-ROOMS PER SQ. FT.	COM-MERCIAL YIELD PER SQ. FT.
	lb.		lb.		lb.	lb.		lb.	lb.		lb.	lb.	lb.
Soybean meal	115	Tannin	30	28	127	2.65	31	151	3.15	41	171	3.56	2.67
"	115	Lignin	45	29	113	2.35	32	139	2.90	42	103	2.15	1.60
Dried brewers' grains	180	"	50	30	127	2.65	33	148	3.08	43	153	3.19	2.40
Brewery sludge	350	None		26	110	2.29	34	148	3.08	44	164	3.42	2.52
Wet brewery yeast	450	"					35	94	1.96				
"	450	Tannin	30	15*	107	2.23	36	146	3.04	46	159	3.31	2.50
Dried blood	60	"	25							47	131	2.73	2.04
"	60	Lignin	40				38	149	3.10	48	125	2.60	1.96
Uramon (42% N as urea)	18	"	47	3†	88	1.84	39	141	2.94	49	140	2.92	2.19
"	18	Tannin	30	4†	94	1.96	40	140	2.92	50	146	3.04	2.29

* No tannin in this plot.
† Composted 42 days out of doors.

Since urea is very toxic to the mushroom mycelium, it may be inferred that a real combination did occur between it and the lignin or tannin. The plot with yeast combined with tannin showed a much better yield than with yeast alone. The probable reason why plots with the brewery sludge alone gave a good yield is because it consists of the proteins of the malt which are precipitated by the tannins of the hops; actually, then, these plots of brewery sludge did contain tannin. Sometimes, under the conditions of indoor composting, fair yields were obtained when no lignin or tannin extracts were used. But usually such plots ammoniated strongly so that the mycelium could not grow, or molds developed and prevented the growth of the mycelium. Indoor composting of synthetic composts requires the use of substances in the *nature* of lignin or tannin and fibrous materials like spent licorice roots or spent tannery bark. The obstacle to the use of this process is that the solutions of tannin and lignin are necessarily acid; although the finished compost has a pH of 7.0 or higher the presence of acidifying substances makes such composts more susceptible to the truffle disease of the mushroom mycelium to be described later.

Preparation of the mushroom bed

In order to obtain good yields it is not only necessary to consider the composition and condition of the compost, but also the quantity used in preparing the mushroom bed. The usual commercial practice is to use about 12 bushels of compost for 24 sq. ft. of bed surface, so that the beds have a depth of about 6 inches after tamping. A licorice root compost, which is an ideal material for experimentation, as already explained, was used to prepare beds in which the number of bushels for 24 sq. ft. of bed surface was varied from 6 to 14 as shown in table IX. The results show that the yield increased in almost the same proportion as the increase in quantity of compost; the yield from 12 bushels was almost double that from 6 bushels. In another experiment the volume or depth of the bed was kept constant by diluting one-half of the root compost with an inert material like spent Quebracho wood chips; the result was that the yield was reduced to about one-half. Thus it appears that the usual commercial practice of making the beds gives the best yields.

In table IX there is shown also how the bed temperatures vary with the quantity of material present in the bed. The bed temperature may be controlled by regulating the quantity of material in the bed. It is necessary to attain temperatures of 140° F. in the beds during the "sweating out process" in order to obtain air temperatures of 125° to 130° F. in the mushroom house for the purpose of killing pests. The plots with 6 to 8 bushels per 24 sq. ft. (table IX) were free of pests because the air temperature was 125° to 130° F. since most of the beds in the mushroom house had 12 bushels, and consequently, a temperature of 140° F. It is evident, then, that about 12 bu. of compost should be used for 24 sq. ft. of bed surface, not only to obtain the best yields but also to obtain the high temperatures necessary for killing pests.

TABLE IX

THE EFFECT OF QUANTITY OF SYNTHETIC LICORICE ROOT COMPOST PER SQUARE FOOT OF BED SURFACE ON THE TEMPERATURE DURING THE "SWEATING OUT PROCESS" AND ON THE YIELD OF MUSHROOMS

PLOT	TEMPERATURES OF "SWEATING OUT PROCESS"		SIZE OF PLOT	BUSH-ELS† FOR 24 SQ. FT.	COMPOST USED WHEN FILLING BEDS, 66% MOISTURE		MUSH-ROOMS FROM 24 SQ. FT.	MUSH-ROOMS PER SQ. FT.‡	DRY WT. BASIS		COMMER-CIAL YIELD PER SQ. FT.
	HIGHEST TEMP. ATTAINED	AV. TEMP. DURING 72 HRS. OF PEAK HEAT*			IN 24 SQ. FT.	PER SQ. FT.			COM-POST PER SQ. FT.	MUSH-ROOMS PER SQ. FT.	
	° F.	° F.	sq. ft.	bu.	lb.	lb.	lb.	lb.	lb.	lb.	lb.
1 / 5	130 / 133	126 / 129	24	6	252	10.5	41 / 39	1.67	3.57	0.167	1.25
2 / 6	135 / 133	130 / 130	24	8	336	14.0	55 / 52	2.23	4.76	0.223	1.65
3 / 7	141 / 139	137 / 136	24	10	420	17.5	58 / 58	2.42	5.95	0.242	1.80
4 / 8	145 / 145	140 / 141	24	12	504	21.0	77 / 76	3.20	7.14	0.320	2.40
9	148	146	24	14	588	24.5	82	3.42	8.33	0.342	2.55
Bed 8			312	8	(336)	(14.0)	53	2.20	(4.76)	0.220	1.65
Pile 5	146	139	1392	12	(504)	(21.0)	78	3.23	(7.14)	0.323	2.40

* Average of six successive readings at 12-hour intervals.
† The average bushel weighed 42 lb.
‡ Average.

pH of the compost and the control of weed and disease fungi

The optimum pH for the growth of the mushroom mycelium is 6.5 to 7.0; this pH range, however, is very suitable for other fungi so that the pH of the compost must be adjusted to prevent weed and disease fungi. The common green molds which are omnipresent on spoiled food, and which have spores that are resistant to high temperatures, are easily prevented from growing on the compost by maintaining the pH above 7.0. The many fungi that can grow on composted manure or synthetic composts when the pH is above 7.0 are all eliminated by the high temperatures attained during the "sweating out process" with the exception of two: Coprinus sp. (chiefly *Coprinus fimetarius*) and *Pseudobalsamia microspora*.

The conditions are ideal for Coprinus sp. when there are sufficient alkaline minerals in the compost to cause a small liberation of ammonia from an abundant supply of nitrogenous matter in the compost and, as a consequence, the pH is 8.0 to 9.0. Ammoniating salts and urea are not as toxic to it as to the mushroom mycelium. The addition of one liter of 50 per cent. ammonium hydroxide to 5 sq. ft. of bed surface during the "sweating out process" encouraged the growth of Coprinus sp., whereas even 10 per cent. NH_4OH prevented the growth of the mushroom mycelium, though there was little change in pH. The control of Coprinus sp. would be achieved by acidifying the compost to pH 7.0–7.5, if this procedure were not favorable to the other heat resistant fungus—*P. microspora*.

Since very little is known about *P. microspora*, which causes the truffle disease of the mushroom mycelium, it is necessary to discuss it in some detail. *P. microspora* cannot grow on a *non-sterile* manure or synthetic compost, even if the compost is acidified so that the pH is reduced to 6.0. It can grow only in the presence of or, more likely, on the mushroom mycelium. The writer (11) has shown that it is one of the few fungi which can produce volatile sulphides. It seems that with the implement of these sulphides this fungus can attack the mushroom mycelium. A quinone-like substance continually volatilizes from the surface of the mushroom mycelium as a result of its intense oxidizing activity; this volatile substance acts as a barrier to all organisms, except insects and *P. microspora*, that attack the mushroon mycelium (11). The sulphides of *P. microspora* enable it to reduce the quinones so that it can attack, or at least grow in the presence of, the mushroom mycelium. The reducing ability of *P. microspora* may be demonstrated when a chemical like alpha naphthol is added to an agar medium and inoculated first with the mushroom mycelium, which causes the agar to be colored purple, and then inoculated with *P. microspora*, which then decolorizes the agar.

The growth of *P. microspora* on the mushroom mycelium is expedited by the presence of acidifying substances and sulphur compounds in the compost, even though the overall pH may be 8.0. The mushroom mycelium produces acids which reduce the initial pH 8.0–8.5 of the compost to 6.0–7.0. It is possible that the presence of acidifying materials in the compost assists the evolution of sulphides by *P. microspora* after the pH of the compost has been

reduced by the mushroom mycelium. It has been observed that the addition of acidifying substances like mineral acids, gypsum, superhosphate, tannic acid, etc., to the compost permits the rapid growth of *P. microspora* on the ensuing mushroom mycelium. When acidifying substances are absent and when the compost is properly buffered with alkaline salts, the mycelium of *P. microspora* is greatly retarded in its growth on the mushroom mycelium and the spores of this organism usually fail to germinate.

The large volatilization of ammonia during the "sweating out process" appears to be an important factor for killing spores of *P. microspora*. The presence of alkaline salts in the compost favors a greater evolution of ammonia. In the attempt by the writer to kill the spores of this fungus by numerous fumigants, such as sulphur dioxide, formaldehyde, chloropicrin, dichloronitroethane, carbon disulphide, etc., only ammonia was found of value. It is necessary, therefore, to prepare synthetic composts with somewhat more nitrogen than is necessary to produce the largest yields in order to insure a sufficient volatilization of ammonia. It is also necessary to add sufficient alkaline salts and adjust the pH at 8.0 to 8.5, not only to assist the evolution of ammonia, but also to build up a high buffering capacity of the compost for reasons previously described. This condition of the compost, however, is highly favorable to the Coprinus sp. It is thus essential to strike a balance: make the conditions in the compost somewhat more favorable to Coprinus than to *P. microspora*, since the former is less dangerous to the mushroom crop. The mushroom mycelium can ultimately grow over the mycelial growth of the Coprinus sp. that preceded it; but when *P. microspora* follows the growth of the mushroom mycelium, the crop is greatly reduced or lost.

Summary

A method is described for preparing synthetic compost for mushroom culture from various source materials of N–P–K to produce about 13 lb. N, 4 lb. P_2O_5, and 10 lb. K_2O in a ton of fibrous material having 70 per cent. moisture, after allowing for the quantities of these three constituents present in the fibrous material. Several thousand tons of synthetic composts have been prepared by this method; it has been tested over a period of 5 years; and the yield has been as good as or better than that from horse manure composts.

One of the chief inadequacies of previous attempts to prepare satisfactory composts or "artificial manures" has been the omission of potash. By the addition of K_2O to synthetic composts prepared from spent licorice roots and brewers' grains, the yield was almost double that of similar composts to which only N and P_2O_5 were added.

The effect of potash, various sources of nitrogen, the subdivision and the character of the fibrous materials, the duration of and conditions for composting, the quantity of compost in the mushroom bed, and the relation of pH to the control of weed and disease fungi on the yield of mushrooms is reported.

A process is described in which tannin and lignin extracts are combined

with nitrogenous materials in order to avoid the lengthy outdoor composting period and to produce greater yields of mushrooms.

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L. F. LAMBERT MUSHROOM SPAWN AND PRODUCTS
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LITERATURE CITED

1. FRIESE, W. Über die Mineral Bestandteile von Pilzen. Zeit. Unters. Lebensmittel **57**: 604–613. 1929.

2. HEIN, I. Straw composts for mushroom culture. Mycologia **22**: 39–42. 1930.

3. LAMBERT, E. B. Normal mushrooms from artificial manure. Science n.s. **70**: 126–128. 1929.

4. ——————. Principles and problems of mushroom culture. Botanical Rev. **4**: 397–426. 1938.

5. RICHARDS, E. H., and HUTCHINSON, H. B. Manufacture of nitrogenous fertilizers and the utilization of nitrogen-bearing solutions. U. S. Patent 1,471,979. 1923.

6. ——————, and ——————. Manufacture of nitrogenous fertilizers. U. S. Patent 1,619,679. 1927.

7. SINDEN, J. W. Synthetic composts for mushroom growing. Pennsylvania Agr. Exp. Sta. Bull. 365. 1938.

8. STOLLER, B. B. Thesis. Iowa State College. 1936.

9. ——————, SMITH, F. B., and BROWN, P. E. A mechanical apparatus for the rapid, high temperature microbial decomposition of fibrous, cellulose material in the preparation of composts for mushroom culture. Jour. Amer. Soc. Agron. **29**: 717–723. 1937.

10. ——————. Synthetic composts for mushroom culture. U. S. Patent 2,260,201. 1941.

11. ——————. Oxidations by mushroom enzymes studied by cultural methods, and the relation of these oxidations to a mechanism for resisting disease. (Unpublished.)

12. WAKSMAN, S. A., and IYER, K. R. N. Synthesis of a humus-nucleus. Soil Sci. **34**: 43–70, 71–80. 1932.

13. ——————, and McGRATH, J. M. Preliminary study of chemical processes involved in the decomposition of manure by *Agaricus campestris*. Amer. Jour. Bot. **18**: 573–581. 1931.

14. ——————, and NISSEN, W. On the nutrition of the cultivated mushroom, *Agaricus campestris*, and the chemical changes brought about by this organism in the manure compost. Amer. Jour. Bot. **19**: 514–537. 1932.

15. ——————, and RENEGER, C. A. Artificial manure for mushroom production. Mycologia **26**: 38–45. 1934.

EFFECT OF BORON IN THE SUBSTRATE ON THE RATE OF NITRATE ABSORPTION AND ON NITROGEN DISTRIBUTION IN NASTURTIUM[1]

GEORGE B. BRIGGS

(WITH ONE FIGURE)

Boron was not considered indispensable for the normal growth and development of higher plants prior to 1923. In this year WARINGTON (32) · proved conclusively that boron was essential for the growth and normal development of *Vicia faba* and several other members of the Leguminoseae. Subsequent investigators, JOHNSTON and DORE (8, 9), McHARGUE and CALFEE (11, 12), McMURTREY (13), SHIVE (24) and others have shown that a small continuous supply of boron is essential for the vegetative growth and reproduction of higher green plants, that the effective range of concentration varies with the species and environmental conditions, and that a deficiency of boron produces characteristic abnormal anatomical and cytological changes within the plant.

The purpose of the experiments here reported was to determine the effect of boron in the substrate on the rate of nitrate absorption and on the distribution of nitrogen in plant tissues.

Golden gleam nasturtium (*Tropaeolum majus,* var. florepleno) was selected for these investigations because it was found in preliminary experiments that this plant responded very rapidly to a deficiency of boron in the substrate (fig. 1).

Methods

Seed of uniform size was selected, disinfected with 1:1000 mercuric chloride for five minutes, thoroughly rinsed in distilled water, and placed between clean moist blotters. When the primary root was 3 cm. long the germinating seeds were placed on paraffined nets stretched over a complete nutrient solution after the method of SHIVE and ROBBINS (25). When the seedling had three leaves, uniform plants were transferred to paraffined cork stoppers (25) which were placed in holes in paraffined tin tops of one-gallon glass candy jars. These culture vessels contained a complete nutrient solution. The plants were continuously aerated and continuously supplied with nutrient solution by the method of SHIVE and STAHL (26). A three-salt stock solution of the following composition was used: KH_2PO_4, 0.0021 M; $Ca(NO_3)_2$, 0.0042 M; and $MgSO_4$, 0.0021 M.

In the complete solution boron, manganese, and zinc were also supplied as boric acid, manganese sulphate, and zinc sulphate, respectively, at the rate of 0.25 p.p.m. of solution. Iron was added as ferric tartrate at the rate of 1 p.p.m. Enough sulphuric acid was added to make the initial pH of the nutrient solution 4.0 to 4.2.

The method of determining absorption rates was essentially that outlined
by CLARK and SHIVE (4). At the end of the absorption interval of nine
hours the plants were separated into leaf-blades, petioles, stems, and roots
and the weight recorded. Representative aliquots of the tissue fractions,
placed in trays, were carefully cut into small pieces and dried at 70° C. in
an oven through which air was rapidly circulating. Dry weights of the
fractions were then determined and the tissue stored in air tight bottles.
The solution in which these plants had been growing and the rinsings of the
necessary supplementary vessels were collected quantitatively and made to
volume. Nitrogen determinations were made on aliquots of this solution in
order to determine the amount of nitrogen remaining in the solution. Since
a known amount of nitrogen was added, the difference between these two

FIG. 1. Characteristic appearance of boron deficient (left) and of normal nasturtium
shoot.

determinations was the amount absorbed by the plants during the nine hour
test period.

Two series of nasturtium plants were used in these experiments. Series
I consisted of thirty-six-day-old plants; series II of fifty-day-old plants.
Previous to the time of selection the plants in both series had received a
complete nutrient solution continuously. This solution was identical with
the solution in which the normal plants were grown during the absorption
studies.

In series I thirty-six-day-old plants were used to study the effect of a
change of boron concentration in the growth media on the rates of nitrate
absorption and nitrogen distribution in young nasturtiums over a relatively
short period of time, fourteen days. Absorption tests were made when these
plants had been on their respective boron treatments for 4, 6, 8, 11, and 14
days.

Fifty-day-old plants were used in series II to study the effect of a change of boron concentration in the growth media on the rates of nitrate absorption and nitrogen distribution in relatively mature nasturtiums over an extended period of time, thirty-one days. Absorption studies were made after the plants had been on their respective boron treatments for 7, 13, 19, 26, and 31 days.

When the nasturtium plants were selected for the absorption tests, one-third were placed in the nutrient solution to which no boron was added (group A); one-third continued to receive the solution containing one-quarter of a part per million of boron (group B); and the remaining one-third were placed in the nutrient solution containing a toxic concentration of boron, fifteen parts per million (group C). Twelve cultures, each containing three plants, were grown at each boron level.

A reduced iron micro-Kjeldahl method was used for the total, and total soluble nitrogen determinations. After the nitrogen was reduced (14), two-tenths of a gram of sodium sulphate was added by means of a dry funnel and 1 ml. of concentrated sulphuric acid containing 12 mg. of selenium oxychloride per ml. was pipetted into the test tube. The sample was then digested and subsequently distilled and titrated.

Dry, finely ground tissue was analyzed for ammonium nitrogen according to the method of PUCHER, VICKERY, and LEAVENWORTH (20).

Nitrate nitrogen determinations were made by using the phenoldisulphonic acid method described by SNELL and SNELL (27).

Results and discussion

ABSORPTION STUDIES

BORON DEFICIENCY.—The data in table I show the milligrams of nitrate nitrogen absorbed in nine hours by nasturtium plants of series I grown at three levels of boron for 4, 6, 8, 11, and 14 days.

It will be observed from a study of this table that a deficiency of boron in the nutrient solution has no immediate effect on the rate of absorption of nitrate nitrogen. Plants grown in boron deficient solutions for more than six days showed a progressive decrease in nitrate absorption, compared with normal plants, with the length of time on treatment. While the total nitrate nitrogen absorbed per culture by boron deficient plants decreased slightly with the length of time of treatment, the total nitrate nitrogen absorbed per culture by normal plants increased very markedly.

The decrease in rate of nitrate absorption by nasturtium plants grown in solutions to which no boron was added increased with the severity of the deficiency symptoms. The external appearance of the tops of nasturtium plants which had been growing for four days in minus-boron solutions were quite normal; the roots, however, were light gray in color compared with the silvery-white color of the normal roots. At the end of the sixth day very definite deficiency symptoms were evident; the terminal growing point of the stem was not developing so rapidly and was a darker green than that of

TABLE I

AVERAGE GREEN AND DRY WEIGHTS AND TOTAL AND AVERAGE RATES OF NITRATE NITROGEN ABSORPTION OF THIRTY-SIX-DAY-OLD NASTURTIUMS OF SERIES I AS INFLUENCED BY BORON CONCENTRATION OF THE SUBSTRATE

DAYS ON TREATMENT	AVERAGE GREEN WEIGHT PER PLANT P.P.M. BORON			AVERAGE DRY WEIGHT PER PLANT P.P.M. BORON			TOTAL NO_3-N ABSORBED PER CULTURE OF 3 PLANTS IN 9 HOURS P.P.M. BORON			NO_3-N ABSORBED PER GRAM DRY WEIGHT IN 9 HOURS P.P.M. BORON		
	0.0	0.25	15.0	0.0	0.25	15.0	0.0	0.25	15.0	0.0	0.25	15.0
	gm.	gm.	gm.	gm.	gm.	gm.	mg.	mg.	mg.	mg.	mg.	mg.
4	6.60	7.30	7.10	0.72	0.73	0.73	14.69	15.34	14.91	6.84	7.11	6.84
6	7.32	8.56	8.86	0.89	0.84	1.01	12.28	15.68	16.97	4.59	6.21	5.58
8	9.26	12.35	10.25	1.18	1.17	1.12	12.74	20.01	16.93	3.60	5.67	5.04
11	9.43	24.00	16.50	1.27	2.22	1.64	11.24	33.70	21.84	2.97	5.04	4.41
14	13.95	34.10	22.50	2.03	3.11	2.19	11.63	38.35	23.00	1.89	4.14	3.51

comparable normal plants. The roots showed definite signs of injury; the large roots were not increasing in length, and the terminal meristems were much darker in color than those of normal roots. In contrast with the long, lateral branch roots characteristic of the normal root system, numerous short, weak laterals developed behind the injured terminal meristems. This lack of development of additional root surface in boron deficient plants and the progressive increase in the extent of the root system of normal plants was reflected in the total absorption per culture during the nine-hour absorption interval. It will be observed from table II that nasturtium plants which were grown for six days in solutions without boron absorbed approximately the same total amount of nitrogen during the nine-hour absorption interval as did plants grown in similar solutions for fourteen days. On the other hand, the total nitrogen absorbed per culture per nine hours by normal plants increased progressively during the period of the absorption studies.

TABLE II

EFFECT OF BORON ON FRESH WEIGHT OF ROOTS AND TOTAL NITRATE NITROGEN ABSORBED BY NASTURTIUMS TRANSFERRED TO BORON TREATMENTS WHEN 36 DAYS OLD, SERIES I

DAYS ON BORON TREATMENT	TOTAL FRESH WEIGHT OF ROOTS PER CULTURE		TOTAL NO_3–N ABSORBED PER CULTURE OF 3 PLANTS IN 9 HOURS	
	P.P.M. BORON		P.P.M. BORON	
	0.0	0.25	0.0	0.25
	gm.	gm.	mg.	mg.
4	3.89	5.73	14.69	15.34
6	4.23	8.10	12.28	15.68
8	5.02	12.02	12.74	20.01
11	4.48	20.20	11.24	33.70
14	6.52	28.50	11.63	38.35

The absorption data for the second series of nasturtium plants are presented in table III. These plants were fifty days old when absorption tests were started and, although they were not growing as rapidly (indicated by the increase in rate of absorption) as the younger plants of the first series, the absorption trends are the same. Deficiency symptoms had appeared in the roots and tops of minus-boron plants at the time the first absorption tests were made. The terminal root meristems of most of the main roots of nasturtium plants which were grown in nutrient solutions without boron for more than eight days were inactive. They soon acquired the characteristic "club-like" appearance as the result of the death of numerous small lateral meristems which in many cases never completely penetrated the cortex and epidermis. The roots of deficient plants were stunted and thicker than normal roots and were a dark gray in color.

Although an absence of boron from the nutrient solution has no immediate effect on the rate of nitrate absorption by nasturtium plants, the growth of normal roots placed in solutions containing no added boron was quickly arrested. There seems to be a close correlation between the subsequent de-

TABLE III

AVERAGE GREEN AND DRY WEIGHTS AND TOTAL AND AVERAGE RATES OF NITRATE NITROGEN ABSORPTION BY FIFTY-DAY-OLD NASTURTIUMS OF SERIES II AS INFLUENCED BY BORON CONCENTRATION OF THE SUBSTRATE

DAYS ON TREATMENT	AVERAGE GREEN WEIGHT PER PLANT P.P.M. BORON			AVERAGE DRY WEIGHT PER PLANT P.P.M. BORON			TOTAL NO$_3$-N ABSORBED PER CULTURE OF 3 PLANTS IN 9 HOURS P.P.M. BORON			NO$_3$-N ABSORBED PER GRAM DRY WEIGHT IN 9 HOURS P.P.M. BORON		
	0.0	0.25	15.0	0.0	0.25	15.0	54.44	54.44	54.44	2.07	2.07	2.07
	gm.	gm.	gm.	gm.	gm.	gm.	mg.	mg.	mg.	mg.	mg.	mg.
0	102.9	102.9	102.9	8.6	8.6	8.6	0.0	0.25	15.0	0.0	0.25	15.0
7	110.8	99.0	100.3	10.8	8.8	9.5	46.33	51.48	49.88	1.44	1.98	1.71
13	140.0	133.3	134.7	17.1	12.7	14.0	43.09	59.82	54.18	0.81	1.53	1.26
19	151.5	165.0	175.2	19.9	14.5	16.8	44.78	65.84	52.92	0.72	1.53	1.08
26	145.0	215.0	189.8	20.0	19.7	19.1	29.40	57.92	49.28	0.45	0.99	0.90
31	123.3	249.4	204.8	16.2*	24.7	24.3	20.41	82.25	62.69	0.45	1.08	0.90

* These plants were slightly inferior at start of experiment.

crease in rate of nitrate absorption and the extent of the root injury since the first measurable deviation from the normal rate of absorption was coincident with the first definite external symptoms of injury to the roots.

Numerous workers (**21, 24, 28, 29, 30, 33**) have recorded the anatomical changes in cells and tissues of the roots of various boron-deficient plants. In all of the plants investigated the meristematic tissues were the first to show the effects of the absence of boron. An early modification of regular mitotic divisions occurs with consequent pathological tissue changes.

Since a deficiency of boron is first evident in those regions of the root in which rapid cytological and histological changes are taking place, it might be logical to assume that the decrease in rate of absorption is a result of the impaired physiological activity of these tissues brought about by an inadequate supply of boron.

PREVOT and STEWARD (**19**) have presented evidence which indicates that those cells which are capable of most active growth attain the greatest degree of salt accumulation. In the normal root these are the cells of the apical segment, the same region which in boron-deficient plants is so completely disorganized.

BORON TOXICITY.—In preliminary experiments it was found that a concentration of 15 parts per million of boron in the substrate produced toxicity symptoms in nasturtiums.

It will be observed from tables II and III that a toxic concentration of boron in the nutrient solution has no immediate effect on the rate of nitrate absorption; and that when plants are grown in solutions containing a toxic concentration of boron, it accounts for the decrease in the rate of nitrate nitrogen absorption.

<div align="center">EFFECT OF BORON ON GREEN AND DRY WEIGHTS AND
MOISTURE CONTENT</div>

It will be observed from table I that, although there was a progressive increase in the average green weight of the plants grown at the three levels of boron during the experimental period of fourteen days, at the end of this time the plants receiving a quarter of a part per million of boron were far superior to those plants grown in deficient and toxic concentrations of boron. When this growth criterion was used, the boron deficient plants were inferior not only to the normal plants but also to the plants grown in solutions containing a toxic concentration of boron. The boron deficient plants made only two-fifths the growth, on the green weight basis, of normal plants. The nasturtiums grown in solutions containing fifteen parts per million of boron made only two-thirds the growth of comparable normal plants during the fourteen days on treatment.

The difference observed in the growth response of boron deficient plants, as measured by the average dry weight per plant, was not as marked as was the case when green weight was used as the growth criterion. The relatively low moisture content of the boron deficient plants (table IV) accounts

for the partial masking of the differences between boron treatments when average dry weight per plant was used as the measurement of growth.

Table III also shows the average green and dry weights per plant of the plants of series II. It is observed from these data that nasturtiums grown in nutrient solutions without added boron for nineteen days were only slightly smaller, on the green weight basis, than comparable normal plants; but on the same basis (green weight production), the plants grown for thirty-one days in nutrient solutions to which no boron was added were much smaller than control plants. The nasturtiums grown in solutions containing fifteen parts per million of boron were only slightly inferior to comparable normal plants at the end of the experimental period (31 days), when average green weight was used as the growth criterion.

TABLE IV

MOISTURE CONTENT OF NASTURTIUMS AS EFFECTED BY THE BORON CONCENTRATION
OF THE SUBSTRATE

		SERIES I					SERIES II		
DAYS ON TREAT-MENT	AGE OF PLANTS	PERCENTAGE MOISTURE			DAYS ON TREAT-MENT	AGE OF PLANTS	PERCENTAGE MOISTURE		
		P.P.M. BORON					P.P.M. BORON		
		0.0	0.25	15.0			0.0	0.25	15.0
	days	%	%	%		days	%	%	%
4	40	89.1	90.0	89.8	0	50	91.7	91.7	91.7
6	42	87.9	90.2	88.6	7	57	90.5	90.6	90.6
8	44	87.3	90.6	89.1	13	63	88.1	89.9	89.5
11	47	86.5	90.7	90.0	19	69	87.4	90.6	90.6
14	50	85.4	90.8	90.3	26	76	86.5	90.8	89.9
					31	81	86.8	90.3	88.2

The low moisture content of the boron deficient plants would account for the slight difference between the normal and boron deficient plants of series II, when average dry weight is used as the growth criterion. There was relatively little difference in the average green and dry weights of nasturtiums grown in solutions containing 0.25 and 15 p.p.m. of boron.

A concentration of 15 p.p.m. of boron in the substrate had a more pronounced effect on the growth of young nasturtiums than on mature plants when average green weights were compared (tables I, III). It is possible that boron accumulated in the leaves of the larger plants to such an extent that the active soluble fraction although still in excess of normal boron concentration was relatively low in the actively synthesizing regions of the mature plants; in the young plants with high absorption rates and a predominance of actively synthesizing cells the soluble boron content was relatively high.

The decrease in percentage moisture and corresponding increase in percentage dry weight, in terms of green weight (of boron deficient plants), would indicate less succulence, less metabolic activity, and the lowest growth

rate in boron deficient nasturtiums in comparison with those grown in solutions to which boron was added. ELTINGE (6) reports in this connection that boron deficient *Zea mays* plants had a higher percentage dry weight than control plants.

SCHMUCKER (22) has suggested that boron exerts some control over the swelling of plasma colloids, at least in actively growing cells. He found that the pollen tubes of certain tropical water lilies (Nymphaea sp.) burst soon after forming in a sugar solution of the same concentration as the nectar on the stigma of the species under investigation. In the stigmatic fluid, of course, they developed normally. He found that the addition of 0.001 to 0.01 per cent. boric acid prevented the bursting of the pollen tubes in the artificial media. In connection with SCHMUCKER's work it is interesting to note that BOBKO, MATVEEVA, and SYVOROTKIN (2), and BERTRAND

TABLE V

MILLIGRAMS OF INORGANIC NITROGEN PER GRAM OF DRY WEIGHT OF 50-DAY-OLD NASTURTIUMS
(SERIES I) GROWN FOR 14 DAYS AT THREE LEVELS OF BORON IN NUTRIENT SOLUTION
CONTAINING NITRATE AS THE SOLE SOURCE OF NITROGEN

PLANT FRACTIONS AND TREATMENT	INORGANIC NITROGEN		
	NH_4	NO_3	TOTAL
	mg./gm.	*mg./gm.*	*mg./gm.*
Leaf blades			
0.0 p.p.m. boron	0.0041	None	0.0041
0.25 p.p.m. boron	0.0039	0.0765	0.0804
15.0 p.p.m. boron	0.0033	0.1640	0.1673
Stems			
0.0 p.p.m. boron	0.0086	0.1173	0.1259
0.25 p.p.m. boron	0.0072	0.8700	0.8772
15.0 p.p.m. boron	0.0023	0.6130	0.6153
Roots			
0.0 p.p.m. boron	0.0351	0.3720	0.4071
0.25 p.p.m. boron	0.0819	1.0600	1.1420
15.0 p.p.m. boron	0.0364	0.6620	0.6984

and SILBERSTEIN (1) found an accumulation of boron in the flowers of apple and *Lilium candidum*, respectively, and that the highest concentrations were found in the stigmas of both flowers.

WARINGTON (33), and SOMMER and SOROKIN (28) have presented some additional evidence which seems to indicate that an initial effect of boron deficiency is a change in the water relations of meristematic cells, as indicated by hypertrophy of cambium cells and periblem tissue of roots. These observations were made on actively growing individual cells and were associated with the first microscopically visible changes resulting from a deficiency of boron.

The data in table IV show that there was a progressive decrease in the percentage of moisture in boron deficient nasturtiums with length of time of treatment, and it is evident that there was a decrease in growth (average green weight per plant) in comparison with normal plants (tables I, III).

These results do not substantiate or disprove SCHMUCKER's suggestion because he was observing the initial changes in individual cells and not the ultimate pathological effects on plant tissues. Boron is not the only element whose absence from the growth medium results in a decreased moisture content; a deficiency of calcium (18) or nitrogen (10, ·15, 16) will produce similar results.

KRAUS and KRAYBILL (10) observed that, in general, there was a close correlation between total nitrogen, nitrate nitrogen, and moisture; and that plants low in total nitrogen and nitrate nitrogen had a low moisture content. They suggested that a rapid growth rate which resulted in the formation of thin walled cells, and an increased percentage of amphoteric

TABLE VI

INORGANIC NITROGEN IN 81-DAY-OLD NASTURTIUMS OF SERIES II GROWN FOR 31 DAYS AT THREE LEVELS OF BORON IN NUTRIENT SOLUTIONS CONTAINING NITRATE AS THE SOLE SOURCE OF NITROGEN, EXPRESSED IN MILLIGRAMS OF NITROGEN PER GRAM OF DRY TISSUE

PLANT FRACTIONS AND TREATMENT	INORGANIC NITROGEN		
	NH_4	NO_3	TOTAL
	mg./gm.	mg./gm.	mg./gm.
Leaf blades			
0.25 p.p.m. boron*	0.0045	None	0.0045
0.0 p.p.m. boron	0.0057	None	0.0057
0.25 p.p.m. boron	0.0043	None	0.0043
15.0 p.p.m. boron	0.0036	None	0.0036
Stems			
0.25 p.p.m. boron*	0.0061	0.561	0.5671
0.0 p.p.m. boron	0.0297	0.181	0.2107
0.25 p.p.m. boron	0.0094	0.426	0.4354
15.0 p.p.m. boron	0.0069	0.078	0.0849
Roots			
0.25 p.p.m. boron*	0.0341	1.550	1.5841
0.0 p.p.m. boron	0.0505	0.597	0.6475
0.25 p.p.m. boron	0.0508	0.966	1.0168
15.0 p.p.m. boron	0.0264	0.427	0.4534

* Analyses made at beginning of experiment when plants were 50 days old.

substances was among the factors which would account for a relatively high moisture content. Since boron-deficient nasturtiums were low in total nitrogen and nitrate nitrogen (tables V, VI), and since a deficiency of boron resulted in a decreased growth rate and the formation of fewer, thin walled cells, the percentage of amphoteric substances whose water holding capacity is relatively large was considerably less than in the rapidly growing vegetative tissues of comparable normal plants. This would indicate that the lower moisture content of boron deficient nasturtiums in series I and II, in comparison with normal plants, was not the direct result of a deficiency of boron itself; it was the result of a change in the nitrogen metabolism and absorption of boron deficient plants associated with an inadequate supply of this element.

TABLE VII

DISTRIBUTION OF NITROGENOUS FRACTIONS IN 50-DAY-OLD NASTURTIUMS OF SERIES I GROWN FOR 14 DAYS AT THREE LEVELS OF BORON IN NUTRIENT SOLUTIONS CONTAINING NITRATE AS THE SOLE SOURCE OF NITROGEN EXPRESSED IN MILLIGRAMS OF NITROGEN PER GRAM OF DRY TISSUE

PLANT FRACTIONS AND TREATMENTS	TOTAL INORGANIC		SOLUBLE ORGANIC		TOTAL SOLUBLE		INSOLUBLE ORGANIC		TOTAL ORGANIC NITROGEN	TOTAL NITROGEN
	mg./gm.	%*	mg./gm.	%*	mg./gm.	%*	mg./gm.	%*	mg./gm.	mg./gm.
Leaf blades										
0.0 p.p.m. boron	0.0041	0.1	0.816	23.4	0.82	23.5	2.67	76.6	3.486	3.49
0.25 p.p.m. boron	0.0804	1.6	1.170	23.5	1.25	25.1	3.72	74.9	4.891	4.97
15.0 p.p.m. boron	0.1673	3.6	1.133	24.1	1.30	27.7	3.39	72.3	4.523	4.69
Stems										
0.0 p.p.m. boron	0.1259	5.1	1.244	50.0	1.37	55.1	1.11	44.9	2.354	2.48
0.25 p.p.m. boron	0.8772	25.4	1.153	33.4	2.03	58.8	1.42	41.2	2.573	3.45
15.0 p.p.m. boron	0.6153	21.3	0.955	33.1	1.57	54.4	1.31	45.6	2.265	2.88
Roots										
0.0 p.p.m. boron	0.4071	11.9	0.743	21.7	1.15	33.6	2.28	66.4	3.023	3.43
0.25 p.p.m. boron	1.1419	21.6	1.128	21.4	2.27	43.0	3.01	57.0	4.138	5.28
15.0 p.p.m. boron	0.6984	14.7	0.922	19.4	1.62	34.1	3.13	65.9	4.052	4.75

* Percentage of total nitrogen.

THE EFFECT OF BORON IN THE SUBSTRATE ON THE DISTRIBUTION
OF NITROGEN IN NASTURTIUM

The experimental data presented in this section represent the analysis of the tissues of fifty-day-old (series I) and eighty-one-day-old (series II) nasturtium plants grown for fourteen and thirty-one days, respectively, in nutrient solutions containing a deficient (0.0 p.p.m. B), a normal (0.25 p.p.m. B) and a toxic (15.0 p.p.m. B) concentration of boron.

BORON DEFICIENCY.—Experimental evidence has shown that in nasturtium plants here employed as well as in other boron-deficient plants carbohydrates accumulate (**9, 24, 29, 30, 31**) and the regions of meristematic activity (**3, 7, 28, 33**) are the first to show symptoms of an inadequate supply of boron. Since rapid protoplasm formation is associated with these regions of active cell division, it appears logical to assume that the pathological phenomena accompanying boron deficiency might very well be associated with a disturbance in the nitrogen metabolism. The analytical data of tables VII and VIII tend to substantiate this assumption.

One of the principal uses of carbohydrates is in protein synthesis. Therefore, if there is little or no external supply of nitrogen (**10, 15, 16**) or if there is a decrease in the rate of synthesis in all or some forms of organic nitrogen (**17**), carbohydrates will tend to accumulate. Although it is evident from the absorption experiments (tables I, III) that boron deficient nasturtiums were very much limited in their ability to absorb nitrates, it must be remembered that the initial decrease in the rate of absorption of plants growing in solutions with no added boron was coincident with the appearance of visible injury to the roots.

A study of table V shows that ammonium accumulated in the leaves of fifty-day-old nasturtiums (series I), which had been growing for fourteen days in minus-boron solutions. In fact, ammonium was the only form of inorganic nitrogen found in the leaves of boron deficient nasturtiums. In normal leaves, on the other hand, ninety-five per cent. of the inorganic nitrogen was nitrate. This might indicate that nitrate was being reduced in the boron deficient nasturtium leaf blades; this reduced nitrogen, however, was not being fully utilized even in the early stages of nitrogen assimilation.

The content of organic nitrogen, both soluble and insoluble, expressed as the percentage of total organic nitrogen, was the same in the leaves of the boron deficient and normal plants; the actual amount in each case, however, was less in the boron deficient plants (table VII). This suggests a lower rate of organic nitrogen synthesis in the boron deficient leaf blades than in those of the control plants.

Ammonium also accumulated in the stems of boron deficient plants although the total inorganic nitrogen content was very low in comparison with normal plants. There was an accumulation of soluble organic nitrogen in the stems of boron deficient plants. There was no appreciable difference in the ammonium content of boron deficient and normal plants but there was an increase in the soluble nitrogen content.

TABLE VIII

DISTRIBUTION OF NITROGENOUS FRACTIONS IN 81 DAY OLD NASTURTIUMS OF SERIES II GROWN FOR 31 DAYS AT THREE LEVELS OF BORON IN NUTRIENT SOLUTIONS CONTAINING NITRATE AS THE SOLE SOURCE OF NITROGEN EXPRESSED IN MILLIGRAMS OF NITROGEN PER GRAM OF DRY TISSUE

PLANT FRACTIONS AND TREATMENTS	TOTAL INORGANIC		SOLUBLE ORGANIC		TOTAL SOLUBLE		INSOLUBLE ORGANIC		TOTAL ORGANIC NITROGEN	TOTAL NITROGEN
	mg./gm.	%†	mg./gm.	%†	mg./gm.	%†	mg./gm.	%†	mg./gm.	mg./gm.
Leaf blades										
0.25 p.p.m. boron*	0.0045	0.10	0.916	21.3	0.92	21.4	3.39	78.6	4.30	4.31
0.0 p.p.m. boron	0.0057	0.26	0.854	39.0	0.86	39.2	1.33	60.8	2.18	2.19
0.25 p.p.m. boron	0.0043	0.10	1.026	23.7	1.03	23.8	3.31	76.2	4.33	4.34
15.0 p.p.m. boron	0.0036	0.10	0.976	26.3	0.98	26.4	2.73	73.6	3.70	3.71
Stems										
0.25 p.p.m. boron*	0.5671	20.10	1.293	46.1	1.86	66.2	0.95	33.8	2.24	2.81
0.0 p.p.m. boron	0.2107	7.00	1.739	58.0	1.95	65.0	1.05	35.0	2.79	3.00
0.25 p.p.m. boron	0.4354	17.21	1.355	53.5	1.79	70.7	0.74	29.3	2.10	2.53
15.0 p.p.m. boron	0.0849	3.84	1.295	58.7	1.38	62.5	0.83	37.5	2.13	2.21
Roots										
0.25 p.p.m. boron*	1.5841	31.06	0.896	17.6	2.48	48.6	2.62	51.4	3.52	5.10
0.0 p.p.m. boron	0.6475	17.90	1.202	33.4	1.85	51.3	1.76	48.7	2.96	3.61
0.25 p.p.m. boron	1.0168	23.21	1.073	24.5	2.09	47.7	2.29	52.3	3.36	4.38
15.0 p.p.m. boron	0.4534	11.99	0.717	18.9	1.17	30.9	2.61	69.1	3.33	3.78

* Analyses made at beginning of experiment when plants were 50 days old.
† Percentage of total nitrogen.

The accumulation of soluble organic nitrogenous compounds coupled with a disturbance in the insoluble organic nitrogen indicates an interruption at some stage in the intermediate synthetic processes before protein formation in boron deficient nasturtiums. Since, however, there was an accumulation of ammonium and carbohydrates, the relatively high content of soluble organic nitrogen in the stems, as compared with check plants, might also indicate proteolysis.

On the basis of analyses of leaf blades and stems of the plants of series I (table VII) it appears that without an adequate supply of boron the synthesis of organic nitrogenous compounds is retarded in the initial stages of the nitrogen assimilatory processes. The analyses of the root tissues do not confirm this.

The distribution of nitrogen in eighty-one-day-old nasturtium plants showing boron deficiency symptoms (table VIII) was essentially the same as in the fifty-day-old boron deficient plants. In all the organs of the boron deficient nasturtium plants of both series except the roots of series I there was an accumulation of ammonium when the boron deficient plants were compared with normal plants on the basis of their ammonium content. The mature plants of series II which were grown for thirty-one days in solutions without added boron showed a higher content of soluble organic nitrogen in the stems and roots and in the leaf blades on a relative basis than in similar organs of control plants. The protein nitrogen was low in the leaf blades and roots of boron deficient nasturtiums in comparison with normal plants. The accumulation of soluble organic nitrogen compounds associated with boron deficiency may be the result of a low rate of protein synthesis. Since, however, this distribution of the organic nitrogenous compounds is more characteristic of the mature nasturtiums of series II, which were grown for an extended period of time (31 days) in solutions without added boron, it is quite possible that the change from normal nitrogen distribution was due to proteolysis.

SHIVE (24) and WADLEIGH and SHIVE (31) have shown, as the result of extensive microchemical studies on boron deficient cotton seedlings, that scattered cells in the tissues of the stem tips became more acid than comparable normal cells as deficiency symptoms increased in severity. Although no ammonium nitrogen was supplied to these plants, ammonium nitrogen accumulated, especially in the more acid cells. They also reported an accumulation of carbohydrates and a progressive degeneracy of the protoplasm as the disease symptoms became more pronounced. JOHNSTON and DORE (9) have presented data which indicate that the accumulation of carbohydrates, especially in the leaves of boron deficient tomato plants, occurs because of injury to the conducting systems. VAN SCHREVEN (29, 30) also suggested that the partial blocking of the conducting systems in tobacco and tomato resulting from injury due to boron deficiency impeded the translocation of starch and sugars. In studying the anatomical changes resulting from boron deficiency in young sugar beet plants, ROWE (21) observed that

callose (callus) plugs occurred much more frequently in the phloem of diseased plants than in the sieve tubes of healthy beets; and that they were sometimes frequent in strands of boron deficient plants which did not exhibit any other abnormalities. She suggests that their development may be the characteristic precursor of the subsequent degeneration in the mature tissue of vascular strands.

The accumulation of both ammonium and carbohydrates and the failure of the cells of meristematic tissues to divide and differentiate normally, strongly suggests that boron is intimately associated with the initial processes of nitrogen assimilation. It is evident, therefore, on the basis of the data presented in this section, that the presence of boron is one of the essential factors in the utilization of (reduced) nitrogen in the assimilatory processes.

BORON TOXICITY.—The distribution of nitrogenous fractions in the tissues of the nasturtium plants of series I and II which showed boron toxicity symptoms are included in tables V, VI, VII, and VIII. These plants were grown in solutions containing 15 p.p.m. of boron.

It is observed from the data in table V that the leaf blades of nasturtiums of series I showing boron toxicity symptoms contained more than twice as much nitrate and less ammonium than did the leaves of comparable normal plants. This accumulation of nitrates suggests that nitrate was not being reduced as rapidly in the leaf blades showing boron toxicity symptoms as in the normal leaves. The fact that no nitrate was found in the leaf tissues of the plants of series II regardless of boron concentration does not necessarily refute this conclusion; these plants were older than the plants of series I and were harvested at a different time of year. Table VII shows that in series I there was a lower content of insoluble organic nitrogen and a higher content of soluble organic nitrogen in the boron toxic leaf blades than in those of control plants; a condition similar to comparable leaf blades of series II (table VIII). These deviations from the normal condition may be due to proteolysis since the leaves showed characteristic boron toxicity symptoms.

Further study of table VII shows that the total soluble nitrogen content of the stems and roots of the boron-toxic plants of series I was lower and that the insoluble organic nitrogen content of the stems and roots of the boron-toxic plants of series I was higher than in those of normal plants. This would suggest that a high concentration of boron accelerates the formation of complex nitrogenous compounds in the stems and roots of nasturtiums.

It is significant that the percentage distribution of the various nitrogenous fractions in the roots of the boron-toxic nasturtiums of series I and II was almost the same (tables VII, VIII). In both cases there was a lower content of total soluble nitrogen and a higher content of protein nitrogen than in control plants. Since root development was slightly retarded in boron toxic nasturtiums and the rate of nitrate absorption was lower than

in comparable normal plants (tables I, III), it is possible that there were fewer young active cells, and that the increase in insoluble organic nitrogen indicated an accumulation of storage protein (**15, 17**).

An examination of the data in tables VII and VIII shows that the total organic nitrogen content of the roots of both normal and boron-toxic plants in series I and of the stems and roots of both normal and boron-toxic plants in series II was nearly the same, but that the total organic nitrogen content of the leaf blades of boron-toxic nasturtiums of both series was lower than in normal nasturtiums. The leaf blades of series II showed the greatest external symptoms of injury due to boron. Although no boron determinations were made, it may be assumed from the work of EATON (**5**) and SCO-FIELD and WILCOX (**23**), that the boron content of the leaf blades showing boron toxicity symptoms was relatively high. EATON found that those tissues which contained the highest concentration of boron manifested the greatest injury. WEBBER (**34**) has come to similar conclusions as the result of anatomical investigations.

It is evident, then, on the basis of the comparison of the data from tissue analysis just presented, that a toxic concentrataion of boron has a definite effect on the accumulation of protein nitrogen.

Summary

1. Nasturtiums grown in nutrient solutions to which no boron was added showed a progressive decrease in nitrate absorption, compared with normal plants, with the length of time of treatment. There was no close correlation between the subsequent decrease in rate of nitrate absorption and the extent of root injury.

2. Ammonium and carbohydrates as well as soluble organic nitrogen accumulated in nasturtiums showing boron deficiency symptoms. It appears, therefore, that in the absence of an adequate supply of boron the amination of carbohydrate derivatives is inhibited.

3. The proportion of total soluble to insoluble organic nitrogen was lower in the stems and roots of plants showing boron toxicity symptoms than in control plants. This suggests an accelerated synthesis of complex nitrogenous compounds.

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LITERATURE CITED

1. BERTRAND, G., and SILBERSTEIN, L. Sur la repartition du bore dans les organes du lis blanc. Compt. Rend. Acad. Sci. **206**: 796–799. 1938.

2. BOBKO, E. V., MATVEEVA, T. V., and SYVOROTKIN, G. S. Récherches sur le rôle du bore dans les plantes. Ann. Agron. **5**: 801–803. 1935.

3. BRENCHLEY, W. E., and THORNTON, H. A. The relation between the development, structure, and function of the nodules of *Vicia faba*,

as influenced by the presence or absence of boron in the nutrient medium. Proc. Roy. Soc. (London) **98**: 373–399. 1925.

4. CLARK, H. E., and SHIVE, J. W. The influence of the pH of a culture solution on the rates of absorption of ammonium nitrate nitrogen by the tomato plant. Soil Sci. **37**: 203–225. 1934.

5. EATON, F. M. Boron in soils and irrigation waters and its effect on plants with particular reference to the San Joaquin Valley of California. U. S. Dept. Agr. Tech. Bull. 448. 1935.

6. ELTINGE, E. T. Effect of boron deficiency upon the structure of *Zea mays*. Plant Physiol. **11**: 765–778. 1936.

7. HAAS, A. R. C., and KLOTZ, L. J. Some anatomical and physiological changes in citrus produced by boron deficiency. Hilgardia **5**: 175–188. 1931.

8. JOHNSTON, E. S., and DORE, W. H. The relation of boron to the growth of the tomato plant. Science n.s. **67**: 324–325. 1928.

9. ——————, and ——————. The influence of boron on the chemical composition and growth of the tomato. Plant Physiol. **4**: 31–62. 1929.

10. KRAUS, E. J., and KRAYBILL, H. R. Vegetation and reproduction with special reference to the tomato. Oregon Agr. Exp. Sta. Bull. 149. 1918.

11. McHARGUE, J. S., and CALFEE, R. K. Effect of boron on the growth of lettuce. Plant Physiol. **7**: 154–161. 1932.

12. ——————, and ——————. Further evidence that boron is essential for the growth of lettuce. Plant Physiol. **8**: 305–315. 1932.

13. McMURTREY, J. E. The effect of boron deficiency on the growth of tobacco plants in aerated and unaerated solutions. Jour. Agr. Res. **38**: 371–380. 1929.

14. MOORE, R. H. A micro-Kjeldahl method including nitrates. Bot. Gaz. **100**: 250–252. 1938.

15. NIGHTINGALE, G. T. Nitrogen nutrition of green plants. Bot. Rev. **3**: 85–174. 1937.

16. ——————, SCHERMERHORN, L. G., and ROBBINS, W. R. The growth status of the tomato as correlated with organic nitrogen and carbohydrates in roots, stems, and leaves. New Jersey Agr. Exp. Sta. Bull. 461. 1928.

17. ——————, ——————, and ——————. Some effects of potassium deficiency on the histological structure and nitrogenous and carbohydrate constituents of plants. New Jersey Agr. Exp. Sta. Bull. 499. 1930.

18. ——————, ADDOMS, R. M., ——————, and SCHERMERHORN, L. G. Effects of calcium deficiency on nitrate absorption and on metabolism in tomato. Plant Physiol. **6**: 605–630. 1931.

19. PREVOT, P., and STEWARD, F. C. Salient features of the root system relative to the problem of salt absorption. Plant Physiol. **11**: 509–534. 1936.

20. PUCHER, G. W., VICKERY, H. B., and LEAVENWORTH, C. S. Determination of ammonia and of amide nitrogen in plant tissue. Ind. & Eng. Chem. Anal. Ed. 7: 152–156. 1935.

21. ROWE, E. A. A study of heart-rot of young sugar-beet plants grown in culture solutions. Ann. Bot. 50: 735–746. 1936.

22. SCHMUCKER, T. Über den Einfluss von Borsäure auf Pflanzen, insbesondere keimende Pollenkörner. Planta 23: 264–283. 1935.

23. SCOFIELD, C. S., and WILCOX, L. V. Boron in irrigation waters. U. S. Dept. Agr. Tech. Bull. 264. 1931.

24. SHIVE, J. W. The adequacy of the boron and manganese content of natural nitrate of soda to support growth in sand culture. New Jersey Agr. Exp. Sta. Bull. 603. 1936.

25. ————, and ROBBINS, W. R. Methods of growing plants in solution and sand cultures. New Jersey Agr. Exp. Sta. Bull. 636. 1937.

26. ————, and STAHL, A. L. Constant rates of continuous solution renewal for plants in water cultures. Bot. Gaz. 84: 317–323. 1927.

27. SNELL, F. D., and SNELL, C. T. A colorimetric method of analysis. D. Van Nostrand Co. New York, 1936. Nitrates. pp. 629–634. 1936.

28. SOMMER, A. L., and SOROKIN, H. Effects of the absence of boron and of some other essential elements on the cell and tissue structure of the root tips of *Pisum sativum*. Plant Physiol. 3: 237–254. 1928.

29. VAN SCHREVEN, D. A. Uitwendige en enwendige symptomen van boriumgebrek bij tabak. Tijdschr. Plantenziek. 40: 98–129. 1934. (English summary.)

30. ————. Uitwendige en enwendige symptomen van boriumgebrek bij tomaat. Tijdschr. Plantenziek. 41: 1–26. 1935. (English summary.)

31. WADLEIGH, C. H., and SHIVE, J. W. A microchemical study of the effects of boron deficiency in cotton seedlings. Soil Sci. 47: 33–36. 1939.

32. WARINGTON, K. The effect of boric acid and borax on the broad bean and certain other plants. Ann. Bot. 37: 629–672. 1923.

33. ————. The changes induced in the anatomical structure of *Vicia faba* by the absence of boron from the nutrient solution. Ann. Bot. 40: 27–42. 1926.

34. WEBBER, I. E. Histological characteristics of plants grown in toxic concentrations of boron. Jour. Agr. Res. 50: 189–194. 1935.

CHANGES IN CERTAIN WATER–SOLUBLE NITROGENOUS CON-STITUENTS OF BURLEY TOBACCO DURING CURING[1]

JAMES R. YOUNG AND R. N. JEFFREY

(WITH TWO FIGURES)

Introduction

An investigation of the chemical changes that take place in tobacco dur-ing curing is in progress at the Kentucky Agricultural Experiment Station. Attempts are being made to determine what the normal changes are and how they are influenced by such variables as temperature and relative humidity, which have been shown to influence the final quality of tobacco. A study of this type adds to the knowledge of leaf metabolism during starvation and drying. Knowledge of the chemistry of curing may lead to methods of pro-ducing high quality tobacco which have not been found empirically.

This paper reports the results of experiments on the changes that occur in certain water-soluble, nitrogenous constituents of the tobacco leaf during curing and of the effect of relative humidity upon these changes in chemical composition. Relative humidity was used as the variable rather than tem-perature because the work of JEFFREY (3) showed that differences in rela-tive humidity cause larger differences in quality of the tobacco than do dif-ferences in temperature, within the limits encountered in practical Burley tobacco curing.

Chemical investigations of the tobacco plant have been made in many regions where tobacco is an important cash crop but very little of this work has dealt with the chemistry of curing. The most complete studies of the chemical changes that occur during the curing of tobacco were made by VICKERY (10, 13) and his associates at the Connecticut Agricultural Experi-ment Station. Primed leaves of a Connecticut shade-grown variety of cigar tobacco were cured in a curing shed and analyses were made of the leaves at different stages of curing. The analyses included insoluble nitro-gen, several fractions of the soluble nitrogen, ash, carbohydrates, and ether extract.

In 1914 GARNER (2), of the Bureau of Plant Industry, reported on some chemical studies of tobacco curing. Both primed and stalk-cured leaves of several Connecticut cigar varieties were analyzed before and after curing. Analyses included several carbohydrate fractions, several organic acid frac-tions, several soluble nitrogen fractions, ash, and protein nitrogen. Ash and dry matter were expressed as grams per 100 leaves. The other analyses were expressed as percentage of dry weight.

Basis for reporting results

Since both the total sample weight and the dry weight of the leaves change during curing, neither of these forms a satisfactory basis for express-

ing results. Some investigators have expressed their findings as weight of component in a given number of leaves. Because of the great variation in size of Burley tobacco leaves, even if taken from the same stalk position, it would be necessary to take a very large sample to make the sampling error negligible. Since it was planned to cure the tobacco in curing chambers under controlled conditions, such large samples could not be used.

VICKERY *et al.* (**10, 13**) working with primed tobacco expressed their results as weight of a component per unit weight of the fresh leaves. Their method could not be used in this study because the tobacco leaves were cured on the stalk. Some investigators have expressed their data as weight of a component per unit area of the fresh leaf. This method is the one used in the present paper as it is possible to estimate the area of tobacco leaves while they are still attached to the stalk.

Methods
DETERMINATION OF LEAF AREA

The length and width of each of the four largest undamaged leaves on each plant were measured to the nearest centimeter before the plants were cut and a tag was attached to each leaf indicating its dimensions. Just before the plants were cut for the curing chambers, the leaves to be used in determining the regression equation relating the product of the length and the width to the area were cut from the plants and the midribs removed. Different methods of determining the relationship between these dimensions and the leaf area were used in the two years in which the study was conducted. In 1939 the lamina (leaf minus midrib) was weighed and plugs of known area were removed by means of a cork borer and weighed. The area of the leaf was calculated by multiplying the weight of the leaf by the ratio of area to weight of the plugs. The constants in the regression equation were evaluated by the method of least squares on data from 42 leaves. The regression equation is:

$$y = \frac{0.76x}{1000} - 0.015$$

where x = the product of the length and width of the leaf expressed in centimeters, and y = the leaf area in square meters. The standard error of estimate σ and the coefficient of correlation r_{xy}, were found to be:

$$\sigma_e = 0.009 \text{ m}^2$$
$$r_{xy} = 0.96$$

Due to the difficulties involved in taking plugs representative of the lamina, a different method was used in 1940. The length and width of the leaves were measured as before but the area was determined by blue-printing the lamina after the midrib had been removed and determining the area of the blueprints by weighing them. The regression equation, standard error of estimate, and the coefficient of correlation found from the data obtained from 100 leaves are:

$$y = \frac{0.65x}{1000} + 0.006$$

$$\sigma_\epsilon = 0.007 \text{ m}^2$$

$$r_{xy} = 0.96$$

Though the constants of these two regression equations are of different magnitude, the lines which they represent cross near the average leaf area so that the results obtained with the two equations are not widely different. Each equation, however, was used in calculating the corresponding year's results.

WOLF and GROSS (15) studied structural responses induced by topping and suckering. They gave data for 50 tobacco leaves which included measurements of length, width, and area. From their data the following regression equation may be calculated:

$$y = \frac{0.63x}{1000} + 0.03$$

$$r_{xy} = 0.99$$

WOLF and GROSS worked with a variety of flue-cured tobacco. One would not expect the regression equation to be the same for different varieties but the magnitude of the coefficient of correlation indicates that there is a high degree of linear correlation between the area of the tobacco leaf and the product of its length and width.

SAMPLING

The analyses were made of leaves from plants of Kentucky Experiment Station Burley no. 16 tobacco grown on the Station farm during the seasons of 1939 and 1940. In 1939 the plants were cut and speared on August 30, but were piled in the field until the next day to wilt. A fresh leaf sample was taken at the time of cutting. The tobacco was housed in the air-conditioned chambers described by O'BANNON (6) and used by JEFFREY (3) in studies of the effect of different air conditions during curing upon the quality of cured tobacco. Since these studies showed that the relative humidity was more critical than the temperature in controlling the quality of the cured tobacco, three different levels of relative humidity were used: one at about the optimum (70 per cent.); one above (78 per cent.); and one below (59 per cent.). The same temperature was maintained in all cases (75° F. or 23.9° C.).

The tagged leaves from the 6 plants on one stick constituted a sample. Since each tier supported 6 sticks it was possible to take one sample from each chamber at each of 6 different times during curing, in addition to the fresh-leaf sample. The time of sampling for each expressed as hours from cutting to sampling is recorded in the first column of table I.

When the samples were taken, the tagged leaves were examined and those damaged in housing were not included in the sample. After the tagged leaves were removed, the rest of the stick of tobacco was returned to the chamber in order to change the conditions surrounding the next sample as

TABLE I

CONCENTRATION OF CERTAIN CONSTITUENTS OF BURLEY TOBACCO LEAF WEB AT DIFFERENT TIMES DURING THE AIR-CURING PROCESS, 1939 CROP, STATI
AS PERCENTAGE OF THE DRY WEIGHT AND AS WEIGHT PER SQUARE METER OF LEAF AREA

TIME	RELATIVE HUMIDITY	DRY MATTER	SULPHATED ASH		TOTAL NITROGEN		NICOTINE NITROGEN		ASPARAGINE NITROGEN		GLUTAMINE NITROGEN		AMMONIA NITROGEN		AMINO ACID N (BY DIFF.)		RESIDUAL NITROGEN	
hr. PFL	%	gm.	%	gm.	%	gm.	%	mg.	%	mg.	%	mg.	%	mg.	%	mg.	%	gm.
0	......	39.6	20.8	8.2	4.5	1.78	0.62	246	......	......	......	......	......	......	......	......	......	1.12
	......	32.3	23.2	7.5	4.5	1.45	0.75	242	0.06	19	0.34	12	0.02	5	0.16	52	3.5	1.12
65	59	35.8	23.6	8.4	4.5	1.61	0.85	304	0.35	125	0.08	27	0.05	17	0.32	114	2.9	0.94
"	70	30.8	23.6	7.3	4.7	1.45	0.88	271	0.28	87	0.07	22	0.04	14	0.39	120	3.0	1.02
"	78	31.9	23.6	7.5	4.9	1.56	0.87	278	0.31	98	0.08	25	0.05	17	0.39	123	3.2	1.02
185	59	28.8	23.4	6.7	3.8	1.09	0.87	251	0.43	124	0.11	30	0.10	28	0.12	36	2.2	0.53
"	70	27.0	24.6	6.6	3.6	0.97	0.85	230	0.38	104	0.07	18	0.11	29	0.17	47	2.0	0.54
"	78	29.0	25.0	7.2	4.2	1.21	0.88	256	0.43	125	0.09	27	0.12	35	0.15	43	2.9	0.72
258	59	26.8	25.2	6.8	4.1	1.10	0.83	222	0.42	113	0.12	33	0.10	26	0.16	43	2.5	0.66
"	70	27.0	23.8	6.4	4.0	1.08	0.87	235	0.53	144	0.07	18	0.11	30	0.15	40	2.2	0.61
"	78	27.2	24.6	6.7	4.0	1.09	0.85	231	0.40	109	0.10	26	0.11	29	0.11	30	2.4	0.67
329	59	27.4	24.6	6.7	4.1	1.12	0.86	236	0.37	102	0.11	30	0.07	20	0.15	41	2.5	0.69
"	70	27.2	24.8	6.7	3.9	1.06	0.82	223	0.38	102	0.07	20	0.12	32	0.13	35	2.4	0.65
"	78	27.5	25.4	7.0	3.8	1.05	0.86	237	0.42	116	0.10	27	0.11	29	0.19	51	2.1	0.59
473	59	27.3	24.6	6.7	4.4	1.20	0.94	257	0.34	92	0.03	7	0.11	31	0.16	42	2.8	0.77
"	70	30.3	24.3	7.4	4.3	1.30	0.94	285	0.46	139	0.17	52	0.10	29	0.03	10	2.6	0.79
"	78	26.4	24.6	6.5	4.0	1.06	0.79	209	0.42	110	0.05	13	0.13	34	0.14	36	2.5	0.66
641	59	27.2	24.0	6.5	4.1	1.11	0.81	220	0.50	135	0.08	21	0.10	27	0.22	58	2.4	0.64
"	70	28.3	26.1	7.6	4.0	1.13	0.87	246	0.41	115	0.04	11	0.12	33	0.14	39	2.4	0.69
"	78	28.2	25.2	7.4	3.7	1.04	0.87	246	0.33	93	0.02	5	0.10	29	0.15	42	2.2	0.63
BC*	......	23.6	25.2	5.9	3.0	0.71	0.43	102	0.37	88	0.05	11	0.10	25	0.19	45	1.9	0.44

* Barn-cured samples.

TABLE II

CONCENTRATION OF CERTAIN CONSTITUENTS OF BURLEY TOBACCO LEAF WEB AT DIFFERENT TIMES DURING THE AIR-CURING PROCESS, 1940 CROP, STATED AS PERCENTAGE OF THE DRY WEIGHT AND AS WEIGHT PER SQUARE METER OF LEAF AREA

TIME	RELA-TIVE HU-MIDITY	DRY MATTER	SULPHATED ASH		TOTAL NITROGEN		NICOTINE NITROGEN		ASPARAGINE NITROGEN		GLUTAMINE NITROGEN		AMMONIA NITROGEN		AMINO ACID N (BY DIFF.)		RESIDUAL NITROGEN	
hr.	%	gm.	%	gm.	%	gm.	%	mg.	%	mg.	%	mg.	%	mg.	%	mg.	%	gm.
0		40.1	21.0	8.4	5.3	2.11	0.72	280	0.05	18	0.03	12	0.02	8	0.18	71	4.5	1.72
32	50	31.7	24.6	7.8	5.8	1.84	0.64	203	0.19	62	0.04	12	0.04	12	0.30	96	4.6	1.46
"	69	36.6	23.1	8.5	5.7	2.07	0.71	262	0.17	62	0.04	16	0.02	9	0.27	100	4.5	1.66
"	86	31.7	24.3	7.7	5.6	1.77	0.71	225	0.20	63	0.06	18	0.03	10	0.31	99	4.4	1.36
68	50	35.0	21.8	7.6	5.0	1.76	0.73	255	0.40	139	0.10	36	0.03	12	0.35	122	3.5	1.20
"	69	34.2	22.8	7.8	5.6	1.92	0.71	241	0.38	131	0.10	36	0.04	14	0.35	121	4.1	1.38
"	86	33.9	22.0	7.5	4.8	1.64	0.65	221	0.33	112	0.11	38	0.03	10	0.36	121	3.4	1.14
102	50	33.0	24.2	8.0	4.9	1.62	0.70	232	0.51	168	0.19	64	0.03	11	0.21	69	3.3	1.08
"	69	30.4	22.9	7.0	5.6	1.70	0.79	241	0.53	161	0.17	52	0.04	13	0.33	100	3.8	1.12
"	86	34.2	24.9	8.5	5.2	1.79	0.77	264	0.44	150	0.16	56	0.05	17	0.29	98	3.2	1.21
169	50	25.7	28.0	7.2	5.2	1.35	0.73	187	0.52	133	0.15	40	0.09	22	0.14	36	3.6	0.77
"	69	34.1	26.4	9.0	5.1	1.74	0.89	305	0.59	200	0.17	58	0.06	19	0.19	64	3.3	1.09
"	86	29.2	25.6	7.5	5.5	1.59	0.76	221	0.53	155	0.15	44	0.06	17	0.31	91	3.7	0.96
236	50	30.0	24.8	7.4	4.2	1.26	0.84	252	0.56	168	0.13	36	0.10	30	0.20	59	2.5	0.71
"	69	28.8	23.0	6.6	4.2	1.21	0.77	222	0.70	200	0.19	54	0.08	23	0.20	59	2.4	0.65
"	86	29.9	26.2	7.8	4.4	1.33	0.84	250	0.44	133	0.11	34	0.10	30	0.17	52	2.8	0.83
504	50	25.0	25.2	6.3	4.9	1.23	0.78	196	0.50	124	0.21	54	0.14	34	0.14	34	3.2	0.79
"	69	28.6	27.0	7.7	5.0	1.42	0.77	221	0.53	150	0.08	22	0.24	69	0.18	51	3.3	0.91
576	86	24.2	31.7	7.7	4.1	0.99	0.40	96	0.08	19	0.06	14	0.05	13	0.02	4	3.5	0.74
BC-1*	....	31.6	26.5	8.4	4.5	1.43	0.90	284	0.42	131	0.11	34	0.14	46	0.12	39	2.9	0.90
BC-2*	....	30.5	27.0	8.2	4.5	1.36	0.89	271	0.42	127	0.07	21	0.12	37	0.10	30	2.1	0.87

* Barn-cured samples.

little as possible.   The midrib was removed from the leaves of the sample
and the web was dried as rapidly as possible in a current of air at 67° C.
The dried leaves were weighed, ground, and stored in waxed paper friction-
top cartons.   Moisture determinations were made and the dry matter per
square meter of fresh leaf was calculated.   LINK (5) found that in plants
dried at 65° C. the only significant alteration of the nitrogenous constituents
was a decrease in the amount of soluble nitrogen due to coagulation.   None
of the substances determined in this study are coagulated by heat.   LINK
did not determine ammonia or amide nitrogen in his study of the effect of
drying.

Harvesting and sampling of the 1940 crop followed the same plan; only
the points of difference will be given.   On September 3, 1940 the plants
were split and cut.   The relative humidity of the three chambers was main-
tained at 50, 69, and 86 per cent.   In 1939, no significant differences were
observed in the composition of the samples cured at the different humidities;
for this reason a wider range of relative humidities was selected for curing
the samples from the 1940 crop.   The results are given in table II.   The
analyses of the 1939 samples indicated that the chemical changes occurred
more rapidly in the early stages of curing.   Therefore most of the samples
of the 1940 crop were taken during the early stages of curing.   At cutting
time two additional sticks were cut after their leaves had been measured as
above and were placed in the barn to constitute the 1940 barn-cured samples.

<center>ANALYSES</center>

DRY MATTER.—The dry weight per square meter of leaf area was calcu-
lated from the leaf area of each sample, and the oven-dry weight of the leaf
web of the sample.

SULPHATED ASH.—One gram of air-dry ground tobacco was weighed into
an ignited and weighed porcelain crucible.   Ten drops of a 5 to 3 solution
of sulphuric acid in water were dropped on the sample.   The sulphuric acid
was added to prevent loss of potash by volatilization.   The crucibles were
placed in a cold muffle which was slowly heated to 700° C.   Moisture was
determined on the samples; and the grams of ash per 100 grams of oven-dry
material (percentage) and the grams of ash per square meter of fresh leaf
were calculated. ˙ A third determination was made if the duplicates did not
agree within 3.5 per cent.

TOTAL NITROGEN.—A semi-micro Kjeldahl method was used.   The diges-
tion was made by a modification of the official GUNNING method of the Asso-
ciation of Official Agricultural Chemists (7).   Fifty milligrams of air-dry,
ground tobacco was introduced into a 100-ml. Kjeldahl flask.   Three ml. of
concentrated sulphuric acid containing 0.1 gm. of salicylic acid were added
and the material was allowed to stand for 30 minutes with occasional shak-
ing.   One-half gram of sodium thiosulphate was added to effect the reduc-
tion of the nitrate nitrogen, and the material was warmed on an electric hot
plate for 5 minutes.   After cooling, 1 gm. of potassium sulphate and a small

piece of selenium were added and the mixture was digested 2 hours. On cooling, 10 ml. of water were added and the digested sample was washed into the KIRK apparatus (4), followed by 6 ml. of 40 per cent. sodium hydroxide. The ammonia liberated was steam distilled into a receiver charged with 5 ml. of 0.1 N sulphuric acid. The excess acid was back titrated with 0.05 N sodium hydroxide. After moisture determinations were made on the air-dry samples by drying duplicate 2-gm. samples for 16 hours in a vacuum oven at 70° C., the nitrogen was calculated as percentage of oven-dry material and as grams of nitrogen per square meter of fresh leaf area. Results were not considered acceptable unless the duplicates agreed within 3 per cent. It was necessary to have the samples finely ground in order to obtain this agreement.

NICOTINE NITROGEN.—Nicotine was determined by the AVENS and PEARCE (1) modification of the silicotungstic acid method. It was necessary to distill about 45 minutes instead of 30 minutes as recommended by AVENS and PEARCE in order to get duplicates agreeing within 2 per cent.

AMIDE NITROGEN.—Glutamine N and asparagine N were determined by the methods of VICKERY, PUCHER, and others (12). The amide N was determined on a hot-water extract of the dry tobacco prepared by heating at 80° C. for 10 minutes (14). In the case of both amides a third determination was made if the duplicates failed to agree within 6 per cent.

AMMONIA NITROGEN.—Ammonia N was determined by the method of PUCHER and co-workers (9). Nicotine interfered with the determination by forming a finely divided crystalline precipitate on reaction with the Nessler's reagent. This interference was overcome by precipitating the nicotine as nicotine silicotungstate following the distillation. The precipitate was removed prior to Nesslerization by centrifuging and an aliquot was taken for the determination. The precipitate could not be removed by filtering as the filter paper contained large enough quantities of ammonia to interfere seriously with the determinations. The excess silicotungstic acid caused no interference.

The nitrogen present as ammonia was calculated as percentage of oven-dry weight and as milligrams of ammonia N per square meter of leaf area. The determination was repeated if the duplicates failed to agree within 5 per cent.

AMINO ACID NITROGEN.—Amino acid nitrogen was determined by the method of PETERS and VAN SLYKE (8) on the hot-water extract of the dry tissue. Ammonia was removed by low-pressure distillation at 40° C. with light magnesium oxide prior to the determination of amino nitrogen in the VAN SLYKE manometric apparatus. A third determination was made when duplicates failed to agree within 1 per cent.

VICKERY and co-workers (12) found that 80 per cent. of the amide nitrogen of glutamine reacted with nitrous acid in the VAN SLYKE manometric apparatus. The total amino nitrogen was calculated by substracting 80 per cent. of the amide nitrogen of glutamine from the amino nitrogen found

by the Van Slyke method.   The amino nitrogen other than that of the amides which will be designated hereafter as amino acid nitrogen was found by subtracting the amino nitrogen of the amides from the total amino nitrogen.   Amino acid nitrogen was reported as the percentage of oven-dry weight and as milligrams of nitrogen per square meter of fresh leaf.

Results

Tables I and II give the results for the 1939 and 1940 crops, respectively. Some of these results are also presented in graphical form in figures 1 and 2, expressed as weight per square meter of fresh leaf.   It should be noted in connection with these tables and figures that the central leaves of the plant, which were analyzed in this work, turned yellow in about 3 to 6 days, and brown in 7 to 12 days.   The low relative humidity samples preceded the higher relative humidity samples in both color changes.

Analyses

DRY MATTER.—A marked decrease in dry matter occurred during the first seven days of curing, after which no significant change was observed. It seems evident that the regular initial sample for the 1939 crop is out of line and that the preliminary fresh leaf sample (designated as PFL) is more nearly correct; consequently, the lines in the figures are connected to this point though both values are recorded.   If this value is used, it may be seen that the loss of original dry matter is about 30 per cent. in each year in which the experiment was conducted.   The preliminary fresh leaf, the initial 1939, and the initial 1940 samples contained 43, 22, and 26 leaves, respectively; thus the initial 1939 sample would be expected to be the least accurate.

VICKERY et al. (10, 13) have reported decreases in dry matter during the curing of the primed leaves of a Connecticut shade-grown variety, amounting to 18 and 20 per cent.   GARNER (2) observed decreases in dry matter of 12 and 15 per cent. during the curing of primed leaves and decreases of 25 to 30 per cent. during the curing of leaves on the stalk.

There are two reasons why stalk-cured leaves might be expected to lose more dry weight than primed leaves.   One is that the stalk-cured leaves do not dry as rapidly as the primed leaves, therefore allowing greater opportunity for catabolic processes to go on which would give rise to volatile compounds.   The other is that there is a chance for translocation to take place from the leaves to the stalk in tobacco cured on the stalk.

It has been found by other workers that, in plants living under adverse conditions, the growing points and meristematic tissue receive soluble nutrients at the expense of older and more mature tissue.   For this reason, translocation might be expected to take place from the leaves to the stalks and thence to the living axillary buds or suckers in tobacco being cured with the leaves still attached to the stalk.   It is possible to remove and plant "suckers" which have been on plants in the curing barn for as much as two months and to obtain good growth.
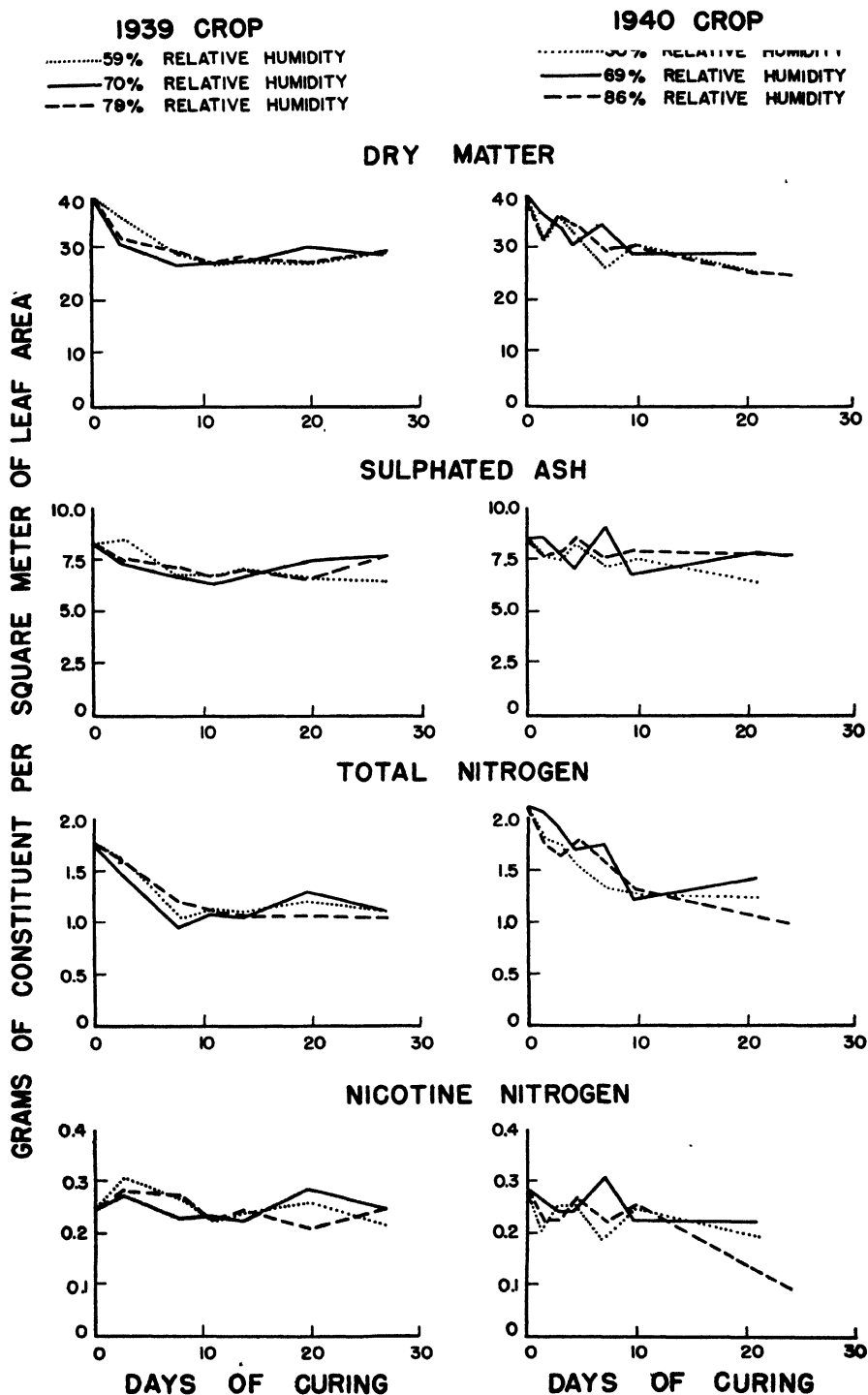
FIG. 1. Changes in the dry matter, sulphated ash, total nitrogen, and nicotine nitrogen content of Burley tobacoo leaf during the air curing process.

FIG. 2.   Changes in the asparagine, glutamine, ammonia, and amino acid nitrogen content of Burley tobacco leaf during the air curing process.

SULPHATED ASH.—The results obtained by this determination are variable.   If the initial samples are averaged and the samples from the later stages of curing are averaged, it may be concluded that the loss in ash content of the leaf web was about 12 per cent.   GARNER (2), however, with fewer samples to indicate the variability of his data, attached significance to a decrease of about 9 or 10 per cent. of the original ash content of the whole leaf.   He found decreases of 3.4 and 4.7 per cent. in the ash content of the leaf web.

TOTAL NITROGEN.—If the preliminary fresh leaf sample is considered to be the most accurate initial 1939 sample, as above, the loss in total nitrogen would appear to be about 38 per cent. in about 8 days in 1939 and about 41 per cent. in about 10 days in 1940.

GARNER (2) observed a larger decrease in total nitrogen in stalk-cured leaves than in primed leaves but he obtained evidence of loss of total nitrogen even in primed leaves; he attributed this to volatilization of ammonia.   The additional decrease in total nitrogen in the case of stalk-cured leaves was attributed to translocation.

VICKERY (10) reported a decrease in total nitrogen in primed leaves of 15 per cent.   This loss occurred before the leaves had all become brown. In a later investigation (13), his data did not indicate any decrease in total nitrogen during the same stages of curing.

NICOTINE NITROGEN.—The nicotine content of the samples expressed on a leaf area basis shows a slight downward trend as curing progresses.   This change is probably too small to be significant except in the final 86 per cent. relative humidity sample; this had undergone "houseburn," which includes attack by microorganisms as well as further autolytic changes by the plant enzymes.   This indicates that nicotine probably did not enter actively into the metabolism of the curing process.   The nicotine values here reported are higher than similar values appearing in the literature for Burley tobacco. No satisfactory explanation can be offered for this difference.   The method used was checked by means of samples previously run by another laboratory that used the A.O.A.C. method, and satisfactory agreement was obtained.

ASPARAGINE NITROGEN.—In 1939 the asparagine content of the curing tobacco increased to almost six times its initial value in the first three days of curing.   After this no significant change was observed.   No significant difference was found between the results at the different relative humidities. In 1940 the asparagine nitrogen increased to over eight times its initial value in about four days and then remained constant except for the 86 per cent. relative humidity sample.   These statements do not imply that there was no further production of asparagine after the third or fourth day, but that the rate of destruction equaled the rate of production if it did continue.   In the 86 per cent. relative humidity sample, the destruction of asparagine apparently continued after the production had ceased, resulting in a value about as low as the initial value.

GLUTAMINE NITROGEN.—The method for the determination of glutamine is rather inaccurate as only small amounts are present and it is calculated

by difference between two determinations neither of which was consistently duplicated within much less than 6 per cent. In 1939, the amount of glutamine nitrogen approximately doubled in the first three days of curing and then held approximately constant until about two weeks from the start of curing, after which it gradually decreased to about the initial value. The result obtained for the sample cured at 70 per cent. relative humidity for 473 hours (19.7 days) cannot be explained except as an experimental error.

In 1940, the glutamine, like the asparagine and to a lesser degree some of the other nitrogenous constituents, reached higher values than in 1939. The glutamine accumulated to over four times the original value and twice the maximum of 1939 in about four days and then gradually fell to about the same value as in 1939 except in the very low humidity sample. If the high final value on this sample is significant, it may be because this sample became very dry while the high glutamine content still existed, inhibiting further chemical change.[2]

The values found for amide content of leaf web were much more variable during the later stages of curing than in the early stages; it does not seem likely that analytical errors are altogether responsible.

VICKERY (10) found that the total amide nitrogen of the hot-water extract of whole tobacco leaves increased from 0.04 gm. per 1000 gm. of fresh leaf to about 0.4 gm. during curing. Later (13), he found that the total amide nitrogen increased from 0.2 gm. per 1000 gm. of fresh leaf to about 1.0 gm. during the same stages of curing.

There are two views concerning the formation of amides in plants. The view of VICKERY (11) is that amide metabolism is a phase of the general respiratory activity of the tissues. The older view of PRIANISCHNIKOW was that the function of amides was to provide a means of disposal of ammonia which otherwise might accumulate in high concentrations and prove toxic. The nitrogen content of the leaves was greater in the 1940 than in the 1939 crop. Also a greater decrease in total nitrogen occurred in the samples from the 1940 crop. This means that probably more catabolic changes occurred which might give rise to ammonia, a form of nitrogen thought to be essential in amide synthesis. A much larger quantity of amides was found in the 1940 samples but this could be due to decomposition in the 1939 samples as previously indicated. If the amount of amides formed in 1940

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[2] Since this work was completed, glutamine has been re-determined on some of the 1940 cured samples. The values did not agree with those previously found; they were so low that they stimulated speculation as to the stability of the amides in air-dry plant tissues when stored for considerable lengths of time. Ten months elapsed from the time the samples were taken from the 1939 crop until these samples were analyzed for amides. Only 4 months elapsed from the time the 1940 samples were taken until they were analyzed for amides. The values for amides of the 1939 crop were much lower than for the 1940 crop and, in view of the values obtained for the 1940 crop nine months after sampling, one is led to the conclusion that the amides may decompose during storage under such conditions. This casts doubt upon the results of this experiment and possibly upon VICKERY's results (14).

was larger because of the increased protein decomposition it still does not prove conclusively whether or not detoxification was actually responsible.

The increase in amide nitrogen appears to have been more immediate than the increase in ammonia nitrogen, particularly in the results from the 1940 crop, where the early samples were taken at more frequent intervals and the analyses are generally more reliable. It may be that the amide nitrogen ceased to rise after about the fifth day because of failure of the supply of α keto acids.

AMMONIA NITROGEN.—During curing of the 1939 crop, the ammonia nitrogen increased to about six times its original value, in the first week, and then remained about constant in all samples. In 1940 the same level was reached after ten days. A high value was obtained for the final 1940 sample at 60 per cent. relative humidity, but this is probably an error. The sample cured at 86 per cent. relative humidity lost ammonia in the later stages of curing, similar to its loss of other soluble nitrogenous constituents.

AMINO ACID NITROGEN.—During curing of the 1939 and 1940 crops, the amino nitrogen other than that contributed by the amides reached a maximum in about three days, after which it decreased, reaching values as low or lower than the initial in another three days in 1939 and somewhat more slowly in 1940. There is probably no other significant change except in the final 1940 high-humidity sample, which is low in amino acid nitrogen as well as other soluble nitrogen components. The hydrolysis of part of the protein to amino acids is one of the first changes that take place in the nitrogen compounds of the curing leaf; this might be expected. It is followed by a rapid conversion of the amino acids to other forms.

During curing of the 1940 crop a decrease of amino nitrogen (other than that of amides) was observed between the third and tenth days, amounting to about 60 mg. per square meter. An increase of two-thirds this amount was observed in the nitrogen of amide groups of asparagine and glutamine. The remainder of the decrease in total amino nitrogen can be accounted for only by translocation or volatilization. During curing of the 1939 crop, a decrease in amino acid nitrogen of about 75 mg. occurred. As practically no net synthesis of amides was detected during this period, the loss probably occurred by volatilization or translocation or both.

RESIDUAL NITROGEN.—If the sum of the asparagine N, glutamine N, ammonia N, amino acid N, and nicotine nitrogen is deducted from the total N, a figure is obtained which is here designated as the residual N. This residual N is probably chiefly protein N and nitrate N. It decreased by about 32 per cent. in the 1939 crop and by about 60 per cent. in the 1940 crop, which had a higher initial nitrogen content. The final value reached is not far different in the two years. In this, as in most other cases, the loss all occurred in the first ten days.

BARN-CURED SAMPLES.—The barn-cured samples were analyzed for the same components as were the samples cured in the curing chambers. The

findings are given in the tables along with the other data, designated BC. The values found agreed, for the most part, with those for the samples cured in the chambers.

Discussion

If we attempt to draw general conclusions from the data presented in the graphs, it will be seen that no significant differences are evident between the tobacco cured at different relative humidities with the single exception of the final sample cured at 86 per cent. This sample was so badly injured that even a person unfamiliar with tobacco would pick it out immediately. The other samples were sufficiently different to be distinguishable by any good tobacco grader. Since the data do not show any such difference, it is obvious that the analyses did not include those substances which are responsible for quality as determined by commercial grade. On the other hand, they did include substances which changed markedly during the curing process. It would appear, as one might expect, that the first change was a hydrolysis of the protein to amino acids which, in turn, were either translocated or yielded ammonia and amides. The increase in ammonia concentration was rather slow at first, but it would appear that ammonia was being produced, as it was probably an intermediate in the formation of the amides. It is also the most volatile nitrogen compound whose presence would be expected. In the 86 per cent. relative humidity sample the loss of all soluble nitrogen compounds was the most complete. Fungus growth was visible on this sample. Probably various microorganisms combined with the plant enzymes to produce the more complete autolysis in this sample.

It is evident that the loss of ash occurred by translocation. Although it cannot be assumed that the movements of inorganic salts and soluble nitrogen compounds would necessarily be the same, the total nitrogen content of the leaves fell by about 40 per cent. while the ash content was falling about 12 per cent.; consequently, it seems possible that some of the nitrogen was lost by volatilization.

After the amide concentration had ceased to rise at about the fourth day, the ammonia continued to rise until about the eighth or tenth day. About this time all significant changes ceased except in the sample which underwent "house-burn." This sample lost additional nitrogen in all soluble forms which were determined, including nicotine. By the end of the curing period, this tobacco was dark colored, had the characteristic odor of "house-burn" known to all tobacco men, and had fungus colonies on it. It is possible that microorganisms may have used the soluble nitrogen compounds in the synthesis of insoluble compounds or undetermined soluble compounds. There was no significant change in the undetermined forms reported in the tables as residual nitrogen. This indicates either that no further attack was made by the plant enzymes or the microorganisms on the leaf protein, or that the amount of protein formed by the microorganisms from soluble sources was about equal to the amount broken down.

Summary and conclusions

This study was made to determine some of the chemical changes that occur in leaves of White Burley tobacco during curing on the stalk, under automatically controlled conditions of temperature and relative humidity. Samples taken at different stages of curing were analyzed for ash, total nitrogen, nicotine nitrogen, asparagine nitrogen, glutamine nitrogen, ammonia nitrogen and amino acid nitrogen. The results are expressed as weight per square meter of fresh leaf. The following conclusions are justified:

1. The dry matter content of the leaf web decreased by about 30 per cent. during the early stages of curing.

2. The total nitrogen decreased by about 40 per cent. of the original amount in the early stages of curing.

3. A rapid increase occurred during the early stages of curing in all forms of soluble nitrogen determined except nicotine.

4. The asparagine, glutamine, and ammonia nitrogen remained high except in leaves cured at very high relative humidity (86 per cent.).

5. The amino acid nitrogen determined by subtracting the amino nitrogen of the amides from the total amino nitrogen decreased from its maximum to below its original concentration. Some of this amino acid nitrogen was used in amide synthesis, some was translocated and some may have been volatilized.

6. The nicotine content remained practically constant throughout the cure except in the "houseburned" sample, indicating that nicotine does not enter actively into the normal metabolism of the curing process.

7. The leaves cured at 86 per cent. relative humidity lost most of their soluble nitrogen during the later stages of curing. This loss is probably due in part to the action of the enzymes of the plant and in part to the action of microorganisms growing on the leaves.

8. Except for the sample cured at 86 per cent. relative humidity, there is no evidence of change in composition after the leaf has burned brown. This indicates that changes due to plant enzymes and microorganisms are both nearly stopped as soon as the tissue has become dry.

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LITERATURE CITED

1. AVENS, A. W., and PEARCE, G. W.   Silicotungstic acid determination of nicotine.   Ind. & Eng. Chem. Anal. Ed. 11: 505–508.   1939.

2. GARNER, W. W., BACON, C. W., and FOUBERT, C. L.   Research studies on curing of leaf tobacco.   U.S.D.A. Bull. 79.   1914.

3. JEFFREY, R. N.   The effect of temperature and relative humidity during and after curing, upon the quality of White Burley tobacco. Kentucky Agr. Exp. Sta. Bull. 407.   1940.

4. KIRK, P. L.   A one piece glass Micro-Kjeldahl distillation apparatus. Ind. & Eng. Chem. Anal. Ed. **8**: 223–224.   1936.

5. LINK, K. P., and SCHULZ, E. R.   Effects of the method of desiccation on the nitrogenous constituents of plants.   Jour. Amer. Chem. Soc. **46**: 2044–2050.   1924.

6. O'BANNON, L. S.   A laboratory for use in determining the influence of various temperatures and relative humidities on the final quality of air-cured tobacco.   Heating, Piping, and Air Conditioning **11**: 699–702.   1939.

7. Official and Tentative Methods of Analysis of the Association of Official Agricultural Chemists, 5th ed.   Washington.   1940.

8. PETERS, J. P., and VAN SLYKE, D. D.   Quantitative clinical chemistry. Vol. II.   Methods.   Williams and Wilkins Company, Baltimore, 1932.

9. PUCHER, G. W., VICKERY, H. B., and LEAVENWORTH, C. S.   Determination of ammonia and of amide nitrogen in plant tissue.   Ind. & Eng. Chem. Anal. Ed. **7**: 152–156.   1935.

10. VICKERY, H. B., and PUCHER, G. W.   Chemical investigations of the tobacco plant.   II.   The chemical changes that occur during the curing of Connecticut shade-grown tobacco.   Connecticut Agr. Exp. Sta. Bull. 324.   1931.

11. ——————, and ——————.   The metabolism of amides in green plants.   II.   The mechanism of amide synthesis.   Jour. Biol. Chem. **128**: 703–713.   1939.

12. ——————, ——————, CLARK, H. E., CHIBNALL, A. C., and WESTALL, R. G.   The determination of glutamine in the presence of asparagine.   Biochem. Jour. **29**: 2710–2720.   1935.

13. ——————, ——————, WAKEMAN, A. J., and LEAVENWORTH, C. S.   Chemical investigations of the tobacco plant.   Carnegie Institute of Washington.   Pub. no. 445, Parts I and II.   1933.

14. ——————, ——————, ——————, and ——————.   Chemical investigations of the tobacco plant.   VI.   Chemical changes that occur in leaves during culture in light and in darkness.   Connecticut Agr. Exp. Sta. Bull. 399.   1937.

15. WOLF, F. A., and GROSS, P. M.   Flue-cured tobacco; a comparative study of structural responses induced by topping and suckering. Bull. Torrey Bot. Club **64**: 117–131.   1937.

A QUANTITATIVE STUDY OF CHLOROSIS IN CHLORELLA UNDER CONDITIONS OF SULPHUR DEFICIENCY

GABRIEL R. MANDELS

(WITH TEN FIGURES)

Introduction

The rôle of chlorophyll in the process of photosynthesis is not clearly understood.[1] Present evidence indicates that under certain conditions there is a direct relation between chlorophyll content and photosynthesis in Chlorella cells (1, 4). On the basis of experiments with intermittent light, EMERSON and ARNOLD (2) suggested the existence of a chlorophyll unit in the light reaction in photosynthesis. Later work has shown, however, that such a relation does not always exist and that the concept of a fixed chlorophyll unit in photosynthesis is no longer tenable (3). It would be of considerable interest to know how the efficiency of chlorophyll changes with varying degree of chlorosis. A thorough investigation of this type is not available. Prerequisite to such a study is a quantitative analysis of the development of chlorosis and of recovery from chlorosis. The present work is an investigation of this nature under conditions of sulphur deficiency.

Methods

The strain of Chlorella used in this investigation was originally isolated from soil by WANN (12) and is known as Chlorella no. 11. It is the same strain that was used in the studies of HOPKINS and WANN (5, 6, 7), FLEISCHER (4), PEARSALL and LOOSE (10), LUDWIG (9), and KENNEDY (8). FLEISCHER'S (4) modification of the nutrient solution employed by EMERSON was used, the composition being: Na citrate—1.00 gm.; KNO_3—1.25 gm.; $MgSO_4 \cdot 7H_2O$—2.46 gm.; KH_2PO_4—1.22 gm.; Fe (as $FeCl_3$)—0.01 gm.; glucose—15 gm.; H_2O—1 l. With the exception of the Na citrate (Baker and Adams, C. P.), (fig. 1) all the chemicals used were J. T. Baker's C.P. analyzed. The sulphur deficient medium was prepared by replacing the $MgSO_4$ with an equivalent amount of $MgCl_2$. Three-liter Florence flasks containing 1.5 l. or 12-l. flasks containing 8 l. of nutrient solution were used as culture vessels. The cultures were sterilized by autoclaving at 15 lb. for 25 minutes or for 35 minutes when the larger vessels were used. The initial pH of the cultures after sterilization was about 5.3. Suspensions of cells grown in a full nutrient solution were employed for inoculation. In some experiments a suspension of cells in sterile distilled water was used, the cells having been rinsed previously in distilled water (three times, by repeated centrifugation and decantation, observing aseptic precautions).

Large culture vessels were used so that the studies could be confined to cultures in which the population density did not exceed 2,000 cells/mm.[3]

[1] Examination of the literature leads to the conclusion that there is little direct data concerning the rôle of chlorophyll.

The possibility of secondary effects due to significant changes in the composition of the nutrient solution was thus minimized.

The cultures were grown in an insulated light chamber at 25 or 30° C. ± 0.5°. A 500-watt tungsten filament bulb placed in a reflector over the top of the chamber served as a light source. Infra-red radiation was filtered out by a ½-inch layer of circulating water. The light intensity was of the order of 500 foot candles as measured on the floor of the chamber with a Weston Illuminometer. The cultures were shaken for 5 minutes every half hour, a mobile platform being in the chamber.

The population density of the cultures was determined with a haemocytometer. A photoelectric colorimeter was used to determine the chlorophyll content of methanol extracts of the cells. Interference by carotenoids was avoided by using a Corning signal red filter having a cut-off at 6300 Å. The colorimeter was calibrated with a preparation of purified cholorphyll extracted from corn leaves following the procedure of SCHERTZ (11). Chlorophyll determinations were always made immediately after removal of the sample from a culture.

EXPRESSION OF DATA

The population density of the culture (N) is in terms of cells per mm.³ of culture. The rate of cell division is the slope of the curve giving the logarithmic increase in cell number per hour, $\left(\dfrac{\Delta \log N}{\Delta T}\right)$. Chlorophyll is expressed in micromoles per 50 ml. of culture, (C), or on a basis of μ moles per 10^9 cells (C_n). The rate of chlorophyll formation is the slope of the curve giving the logarithmic increase in chlorophyll, $\dfrac{\Delta \log C}{\Delta T}$.

Results

DEVELOPMENT OF CHLOROSIS IN SULPHUR-DEFICIENT CULTURES

Practically no growth will occur if a suspension of washed cells, grown in a full nutrient solution, is used to inoculate a culture to which no sulphate has been added. Thus neither previous accumulation of sulphur in cells nor sulphur impurities in the chemicals used, are significant. In the experiments reported here, sufficient sulphate was added to support growth up to a population density of the order of 2,000 cells per mm.³

Data showing typical behavior during growth in a sulphur deficient culture are presented in figure 1. It is seen that cell division and chlorophyll formation gradually cease. It is significant that the rate of chlorophyll formation decreases more rapidly than the rate of cell division. Thus there is an initial decrease in the amount of chlorophyll per cell while chlorophyll formation is still occurring. Even after cessation of chlorophyll formation there is a slight increase in cell number. This results in a further decrease in the chlorophyll content per cell. Development of chlorosis continues due to decomposition of chlorophyll in the cells. Decomposition is initiated about the time cell division ceases.

To get a quantitative measure of the degree of chlorosis the chlorophyll deficit of the chlorotic cells can be expressed as a percentage of the normal chlorophyll complement.

$$\text{Percentage of chlorosis} = \frac{C_{N_0} - C_{N_1}}{C_{N_0}} \times 100$$

where $C_{N_0} = \mu$ moles chlorophyll/10^9 normal cells $= 3.50$; $C_{N_1} = \mu$ moles chlorophyll/10^9 chlorotic cells. On this basis it is found that when chlorophyll synthesis stops, the cells are 45 per cent. chlorotic. The slight increase in cell number found after this point results in 64 per cent. chlorosis when cell division stops. These values for the degree of chlorosis at cessation of chlorophyll formation and at cessation of cell division appear to be quite
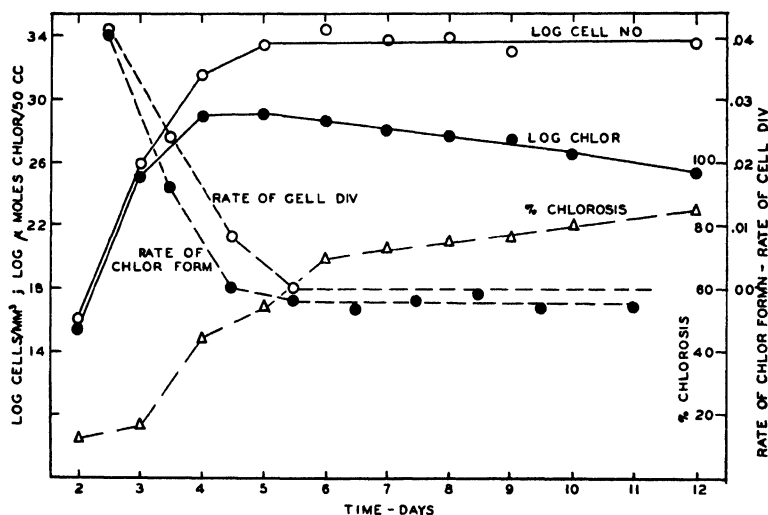
FIG. 1. Growth in culture lacking sulphur showing the development of chlorosis at 25° C. (The ordinate log. chlorophyll has been shifted up 4.75 units.)

constant. Decomposition of chlorophyll results in a gradual increase in the degree of chlorosis. Complete loss of chlorophyll (100 per cent. chlorosis) is attended by death of the cells since they no longer respond to addition of sulphate. Such cells contain significant amounts of carotenoids. The carotenoids decompose gradually in the dead cells.

Microchemical tests of the chlorotic cells with Sudan IV in lactophenol and with I–KI show an accumulation of considerable amounts of fat and little or no starch. This behavior is also found if sugar is omitted from the medium. Normal cells in full nutrient solution do not contain sufficient fat or in a form to be demonstrated with Sudan IV.

RECOVERY FROM CHLOROSIS

To follow the process of recovery an aliquot of a sterile, standard solution of K_2SO_4 was added aseptically to cultures containing sulphur deficient, chlorotic cells. Samples were removed aseptically at intervals for chloro-

phyll determinations. In some experiments lasting only about 24 hours, aseptic precautions were not observed. In such cases bacterial or fungal growth was insufficient to have any noticeable effect. The cultures were kept in the growth chamber during recovery.

Chlorophyll formation is the first visual evidence of recovery—(macro- or microscopic). This can be detected about five hours after the addition
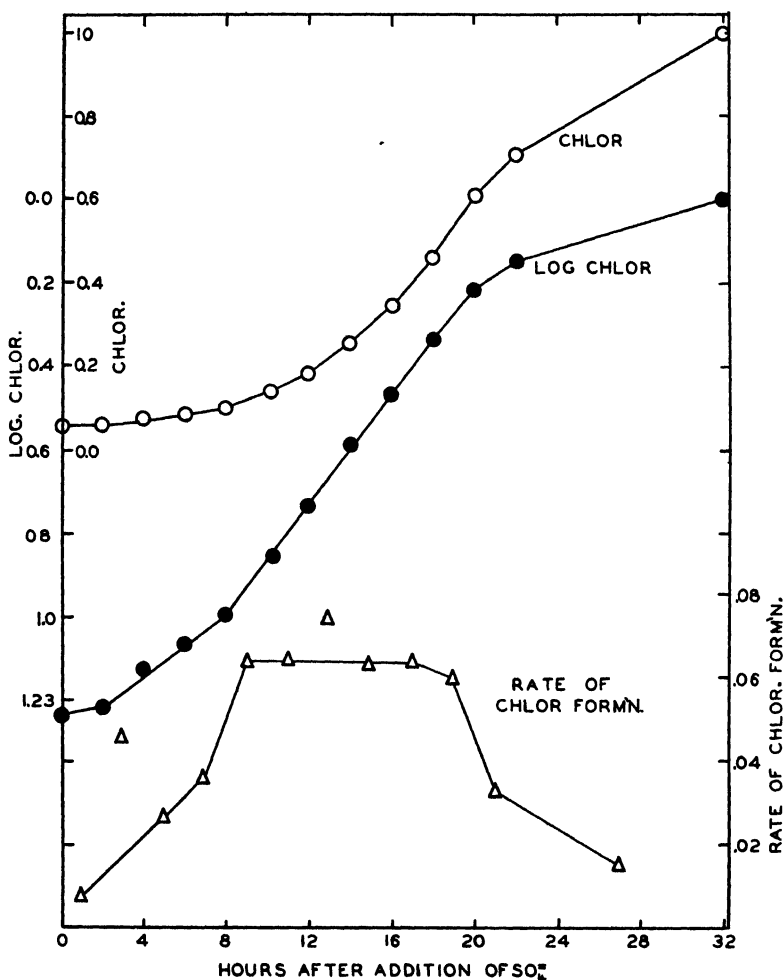
FIG. 2. Chlorophyll formation during recovery from sulphur deficiency. (2090 cells per mm.³; 84 per cent. chlorosis; 1.67 p.p.m. of S as $SO_4^=$; 25° C.)

of sulphate. In a culture having a population of 1500 cells/mm.³, formation of chlorophyll occurs at concentration of sulphate as low as 3×10^{-7} M (0.01 p.p.m. of S). Recovery does not proceed sufficiently to permit cell division unless about 0.1 p.p.m. of S is added. If cell division is to occur there is a lag of about 24 hours before autospore formation is noted. Growth by increase in cell volume, as noted by microscopic observation, occurs before the cells divide (no quantitative measurements were made).

Typical data showing the formation of chlorophyll are presented in figure 2. The process of chlorophyll formation during recovery can be separated into four phases:

1. A lag period of a few hours (usually about four hours) between addition of sulphate and chlorophyll formation.

2. A period of acceleration in chlorophyll synthesis during which the rate of formation increases from zero to a maximum which is attained at about the tenth hour.

3. A period of logarithmic increase in chlorophyll. It should be noted that the maximum rate of synthesis which is maintained during this period (about 10 hours) is considerably in excess of the rate of chlorophyll formation during normal growth in full nutrient solution.
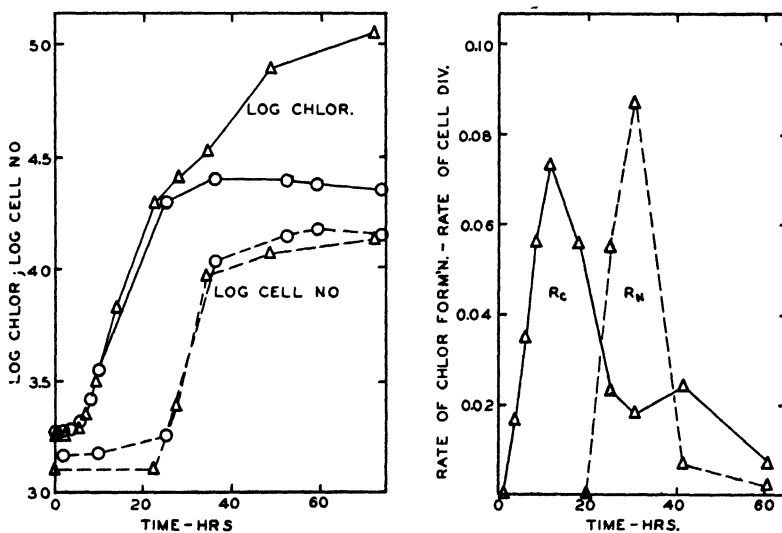
FIG. 3. Chlorophyll formation and cell division during recovery. (1.93 p.p.m. of S ◯; 10.0 p.p.m. △; broken line — log. cell no.; solid line — log. chlorophyll) (70 per cent. initial chlorosis; 25° C.)

FIG. 4. Rate of chlorophyll formation (R_c) and rate of cell division (R_N) during recovery in 10 p.p.m. of S culture.

4. A period of decrease in rate of synthesis. In the culture under consideration here, sufficient sulphate for continued recovery was not present so that the rate decreases to zero.

Data showing recovery with respect to both chlorophyll formation and cell division in two comparable cultures at different concentrations of sulphate are given in figures 3 and 4. There is a rapid increase in chlorophyll in both cultures. The cells, initially about 70 per cent. chlorotic, contain several times the normal chlorophyll complement at the time cell division is initiated. At a concentration of 1.93 p.p.m. of S there was insufficient sulphate for continued recovery. Thus there is only a slight increase in chlorophyll after the inception of cell division. A rapid decrease in the amount

of chlorophyll per cell is found during the spurt in cell division (from 8.66 to 1.88 μ moles/10^9 cells). Subsequently there is a gradual decrease due first to cell division and finally to decomposition. Thus the cells have returned to a condition of sulphur deficiency.

At the concentration of 10 p.p.m. further recovery was possible, synthesis of chlorophyll continuing after the initial spurt in cell division. The sudden decrease in slope of the log chlorophyll curve at the time cell division starts should be noted (fig. 4).

It should be observed that while there is a relation between the amount of chlorophyll formed and the amount of sulphate added, there is none with respect to increase in cell number. The chlorophyll yield will be considered in more detail later.

Since a detailed analysis of the process of cell multiplication during recovery is not directly pertinent to the scope of this paper, a brief summary will be adequate. Although chlorophyll formation can be detected when as little as 0.01 p.p.m. of S is added to a suspension of chlorotic cells, at least ten times this amount must be added before recovery proceeds to a stage where cell division will occur (in a culture of 1500 cells/mm.³). With this minimum amount of sulphate there will be a nine-fold increase in cell number. Increasing the sulphate concentration ten times above the minimum for cell division will still result in the same increase in cell number. If still greater amounts of sulphate are added then there will be an initial nine-fold increase in cell number followed by a short lag before further cell division continues. Thus at least at low concentrations of sulphate the increase in cell number is not proportional to the amount of sulphate added.

FACTORS INFLUENCING CHLOROPHYLL FORMATION DURING RECOVERY

EFFECT OF INITIAL DEGREE OF CHLOROSIS.—To study the effect of length of exposure of cells to a condition of sulphur deficiency upon subsequent recovery, a culture containing 8 l. of nutrient solution was used. Samples were removed aseptically at intervals corresponding to 6.83, 8.88, and 12.21 days after inoculation. Chlorophyll formation was studied after adding 1.0 p.p.m. of S as $SO_4^=$. Thus recovery was followed using portions of the same suspension of cells having chlorophyll complements equivalent to 22, 28.5 and 45 per cent. chlorosis. Although the first sample of cells was only 22 per cent. chlorotic, cell division had ceased. This is contrary to the preceding data in which cell division ceased when the cells were about 65 per cent. chlorotic. This anomalous behavior was probably due to inadequate provision for gas exchange during the development of the culture. Presumably the low O_2 tension suppressed the amount of growth by cell division while chlorophyll formation did not suffer similar interference. The results reported below have been substantiated using "normal" sulphur deficient cells.

The data are summarized in figures 5 and 6. During the first few hours after addition of sulphate there is a decrease in chlorophyll. The extent of

this decomposition is greatest in the slightly chlorotic 'cells and decreases progressively in the two subsequent series. The interpretation of this phenomenon is not clear. The rate is too great to be normal decomposition.

The recovery curves (fig. 5) show that despite the relatively large differences in initial chlorophyll content the maxima reached are practically the same in all three series. Thus the number of moles of chlorophyll formed per mole of sulphate added is determined to a slight extent by the initial degree of chlorosis, the exact figures being 0.307, 0.356, and 0.360 for the three series.

Consideration of the rate of chlorophyll synthesis in the three series brings out another interesting relation (fig. 6). The curves show that the maximum rate of synthesis attained in each of the series is higher as the initial degree of chlorosis increases.
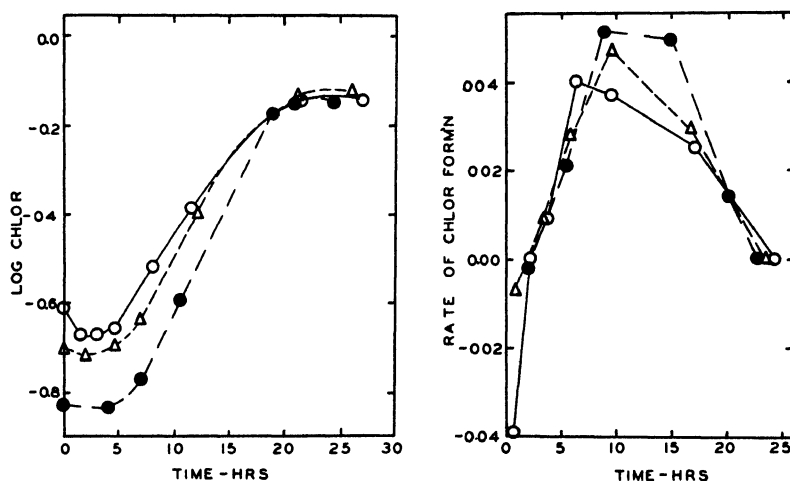
Fig. 5 and 6. Effect of initial degree of chlorosis on chlorophyll formation during recovery. (○ — 22 per cent.; △ — 28.5 per cent.; ● — 45 per cent. chlorosis.)   (1.0 p.p.m. of S; 1520 cells 1 mm.³; 25° C.)

EFFECT OF SULPHATE CONCENTRATION ON THE RATE OF CHLOROPHYLL SYNTHESIS.—Eight liters of a suspension of sulphur deficient cells were divided into equal portions to which different amounts of sulphate were added. The vessels were then placed in the growth chamber under a condition of darkness to minimize chlorophyll decomposition. Since identical suspensions of cells were used, sulphate concentration was the only variable. Data showing the changes in rates of synthesis in the cultures are summarized in figure 7. Two effects are to be observed. First, the effect on the acceleration of chlorophyll synthesis—increasing the concentration up to about 0.5 p.p.m. of S results in a more rapid increase in rate of synthesis. Greater increments have little or no effect (see also fig. 3). Secondly, it is seen that the maximum rate of synthesis attained increases as sulphate concentration is raised to about 0.5 p.p.m. of S. This relation is seen more exactly in figure 8. From 0.05 to 0.5 p.p.m. the relation $R_c = K \log [SO_4^=]$ obtains (where $R_c =$ maxi-
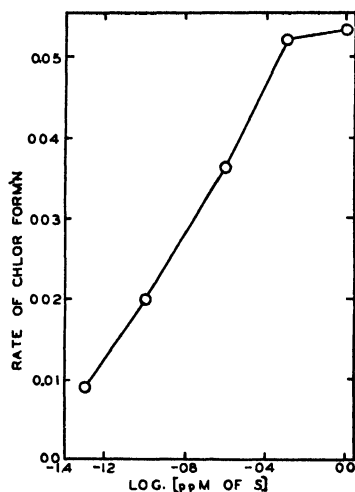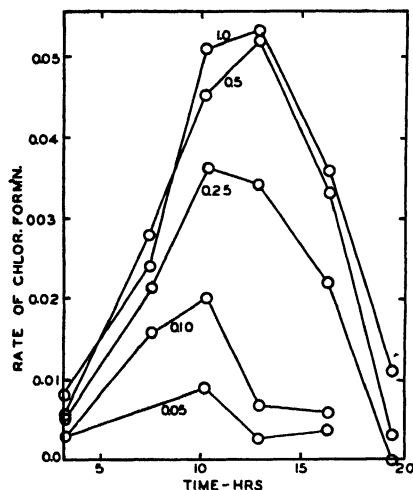
FIG. 7. Effect of sulphate concentration (figures in p.p.m. of S) on chlorophyll formation during recovery.

FIG. 8. Maximum rate of chlorophyll synthesis attained at different concentrations of sulphate during recovery.

mum rate of chlorophyll formation). At concentrations greater than 0.5 p.p.m. there is little or no effect on the maximum rate (see also fig. 3).

EFFECT OF LIGHT.—One of two similar cultures was darkened by enclosing it in several layers of black cloth with black rubberized cloth on the outside. Both cultures were then placed in the light chamber after adding sulphate. Any temperature differences were not considered as being significant. The results are shown in figures 9 and 10. Although the differences between the
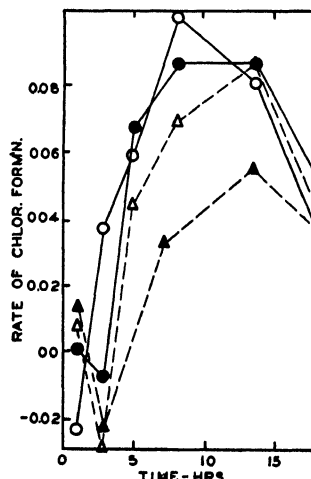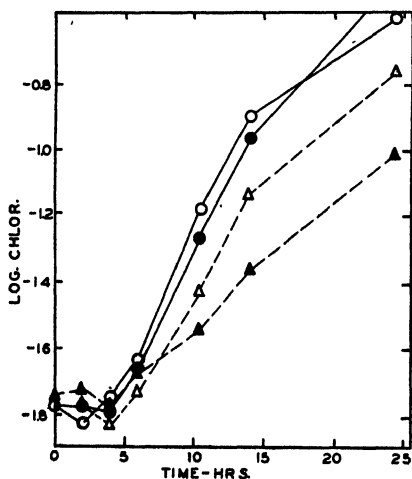
FIG. 9. Effect of light and source of sulphur on chlorophyll formation during recovery. ($\bigcirc$ — $SO_4^=$ in light; $\bullet$ — $SO_4^=$ in dark; $\triangle$ — H_2S in light; $\blacktriangle$ — H_2S in dark.)

FIG. 10. Effect of light and source of sulphur on the rate of chlorophyll formation. (Symbols same as in fig. 9.)

two treatments are not very great, it should be noted that there is a slightly longer lag in the dark and also that the maximum rate of chlorophyll synthesis attained is slightly less in the dark. Another effect is brought out in table I which shows the chlorophyll yield to be slightly greater in the dark. Limited data show that during recovery the rate of cell division is retarded to a much greater extent in the absence of light than is synthesis of chlorophyll.

SOURCES OF SULPHUR AVAILABLE.—Some qualitative studies were made to test the availability of different sulphur compounds for recovery from

TABLE I

MOLES CHLOROPHYLL FORMED* DURING RECOVERY PER MOLE OF SULFATE ADDED

SULPHATE CONCENTRATION		MOLES $SO_4^=$ ADDED/10^9 CELLS	INITIAL CHLOROSIS	CELL DIVISION	MOLES CHLOR. FORMED/MOLE $SO_4^=$ ADDED
			%		
Light	1.93 p.p.m. of S	42.0	70.5	+	0.310
	1.0	20.4	22.0	+	0.307
	1.0	20.4	28.5	+	0.356
	1.0	20.4	45.0	+	0.360
	1.0	24.4	68.6	+	0.394
	0.5	10.2	22.0	+	0.314
	0.5	10.2	28.5	+	0.357
	0.5	10.2	45.0	+	0.415
	0.167	2.74	85.4	−	0.291
	0.10	2.44	68.6	−	0.321
	0.01	0.244	68.6	−	0.321
Dark	1.0	20 4	52.0	?	0.280
	0.5	10.2	51.8	?	0.405
	0.25	5.1	51.8	?	0.483
	0.10	2.04	51.8	?	0.483
	0.05	1.02	51.8	?	0.420
	0.05	1.02	51.8	?	0.385
	0.025	0.51	51.8	?	0.456

* Amount of chlorophyll formed = max. chlorophyll during recovery – initial chlorophyll.
 Average yield in light—0.34 moles chlor./mole sulphate.
     "   "   " dark—0.42   "   "   "   "

sulphur deficiency. All the organic compounds tested were unavailable (cysteine, glutathione, methionine, thioglycollic acid, thiamin, potassium thiocyanate). The possibility that toxic concentrations of these substances were used is unlikely since recovery occurred in control cultures to which both sulphate and the various compounds were added. All of the inorganic compounds tried were available for recovery (hydrogen sulphide, thiosulphate, pyrosulphate, sulphite, persulphate). Tests also showed that sulphate could not be replaced by tellurate or selenate.

While it would be very interesting to have quantitative data showing the length of the lag period, the rate of chlorophyll synthesis and the chlorophyll yield during recovery when different sulphur compounds were used, comparative studies were made only with H_2S and $SO_4^=$. The results of this study are summarized in figures 9 and 10. The curves show a longer lag

when sulphur is supplied as H_2S than as sulphate and also a slower rate of synthesis, these effects being more pronounced in the dark.

EFFECT OF TEMPERATURE.—No detailed study was made of the effect of temperature on chlorophyll formation during recovery. Data are available, however, to show that: (1) The maximum rate of synthesis attained at 30° C. is significantly higher than at 25° C. (2) Recovery does not occur (chlorophyll formation or cell division) at 10° C. or lower, although the cells are not permanently injured by this treatment.

NECESSITY OF OXYGEN.—Results of several experiments showed that no recovery occurs if the atmosphere in equilibrium with the culture medium was unpurified tank nitrogen (probably containing about 0.5 per cent. O_2).

CHLOROPHYLL YIELD DURING RECOVERY.—It is obvious that the extent of recovery must be some function of the amount of sulphate added and data presented already have anticipated the conclusion that the amount of chlorophyll formed shows some proportionality to the amount of sulphate added. Results from several different experiments showing the amount of chlorophyll formed during recovery per mole of sulphate added are summarized in table I. A consideration of the data obtained during recovery in the light shows that the chlorophyll yield is more or less constant over a wide range in concentration of sulphate—from 0.01 to 1.93 p.p.m. of S. About 0.34 moles of chlorophyll is formed per mole of sulphate added. In the dark, the yield is slightly greater, being 0.42 moles of chlorophyll per mole of sulphate added.

Discussion

Presumably the pattern of development of chlorosis is distinct with deficiencies of various essential elements which result in chlorosis. In the case of sulphur deficiency, chlorosis develops in two stages. The first phase is due to the development of a differential between the rate of chlorophyll formation and the rate of cell division, chlorophyll synthesis decreasing before cell division. The second phase is due to decomposition of chlorophyll in the cells and is initiated at about the time cell division ceases. Attention should be directed to the fact that both cell division and chlorophyll synthesis do not stop abruptly as the supply of sulphur becomes limiting. There is a gradual decrease in rate. This can be interpreted in two ways. It is conceivable that this is not real but is only an apparent, statistical effect; the individual cells in a culture being of different ages would not all show deficiency symptoms at the same time. Microscopic observation reveals this to be at least partially true. On the other hand, it is possible that the phenomenon is real—that as the concentration of available sulphur decreases to some minimum value, the rate of chlorophyll synthesis, etc., gradually decreases. If this is true, it follows that the rate of cholorphyll synthesis and other metabolic processes are determined, at least partially, by the concentration of sulphate when it becomes limiting. In the author's opinion the true interpretation of the gradual decrease in rate is to be found by combining these two explanations. Assuming that the rate of chlorophyll syn-

thesis and other metabolic processes decrease gradually as sulphate becomes limiting, it follows that the rates of these processes are functions of sulphate concentration under these conditions. In this connection it is significant to note that with a decreasing supply of sulphate there is a differential action upon at least some metabolic processes. More specifically, chlorophyll formation is more sensitive to a deficiency of sulphur than is cell division. Differential responses of this nature could be useful in studies of sulphur metabolism.

Further evidence illustrating a disturbance of cell metabolism in sulphur-deficient cells is the observation that these chlorotic cells contain large quantities of fat, and little or no starch. This fat accumulation also occurs when the cells are grown autotrophically. (Starch is the normal storage product in Chlorella, very little or no fat being found.)

Addition of sulphate to sulphur-deficient cells was found to result in rapid recovery from chlorosis. When sulphate is added in concentrations of 0.5 p.p.m. of S, or greater, the recovery curve showing the formation of chlorophyll can be separated into four phases: (1) a lag period; (2) a period of increase in rate of synthesis to a maximum; (3) a period of exponential increase in chlorophyll at the maximum rate; and (4) a decrease in rate of synthesis to zero or to normal depending upon the amount of sulphate available.

It is conceivable that the initial lag is due to slow penetration of sulphate. This would appear very unlikely. If slow penetration were responsible there should be some direct relation between concentration of sulphate and length of the lag period since, according to Fick's law, the rate of diffusion is directly proportional to the concentration gradient. No such relation was found. If this lag is a real phenomenon, then it is to be inferred that sulphate bears no simple, direct relation to the synthesis of chlorophyll. It is possible that sulphate is metabolized during the lag period, these anabolic processes culminating or being involved in the synthesis of chlorophyll. On the other hand, it is conceivable that free sulphate initiates or activates a series of reactions leading to chlorophyll formation. It would seem that the first of these postulates is more reasonable in view of the stoichiometric relation between sulphate added and chlorophyll formed.

The initial degree of chlorosis—that is, the degree of sulphur starvation—has some bearing on the behavior during the lag period. The effect cannot be stated in any more definite terms because of insufficient data. It should be pointed out, however, that significant decomposition of chlorophyll occurs during the first few hours after addition of sulphate to slightly chlorotic cells.

An interesting phase of the formation of chlorophyll during recovery is the exponential increase in chlorophyll. The slope of the line showing the logarithmic increase in chlorophyll is several times (2.6) greater during recovery than during normal growth in full nutrient solution. It would appear from this that the factor (or factors) normally limiting the rate of

chlorophyll synthesis is not in operation during recovery from chlorosis. It should be pointed out in this connection, that with increased initial chlorosis the rate of logarithmic formation of chlorophyll during recovery was found to be greater. Although several interpretations of the behavior are possible the information available is inadequate to make discussion profitable.

The relation between the amount of sulphate added to a suspension of chlorotic cells and the amount of chlorophyll formed is significant. Summaries of data from several experiments reveal that about 0.34 moles of chlorophyll are formed per mole of sulphate added over a concentration range of 0.01–1.93 p.p.m. of S. This independence of the chlorophyll yield upon concentration of sulphate would indicate complete absorption of sulphate. The existence of this stoichiometric relation would seem to preclude any direct or indirect catalytic action of sulphate.

With greater initial chlorosis the chlorophyll yield is slightly increased. It should be mentioned that the increased yield is the same at different concentrations of sulphate and is equal to the differences in the initial amount of chlorophyll present in the cells. Thus if a given amount of sulphate is added to suspensions of cells of different degrees of chlorosis the maximum chlorophyll content which is found in the different suspensions is the same. An obvious hypothesis to interpret this behavior would be that decomposition products accumulate in the cells when the degree of chlorosis increases due to chlorophyll decomposition and that synthesis of chlorophyll from these products can occur during recovery without utilization of sulphate. This hypothesis must be discarded, however, since more rigid consideration of the data do not lend support.

For many years, it has been known that Chlorella can form chlorophyll when grown in the dark. It has also been recognized that when cultured in the dark on glucose, Chlorella grows much more slowly than in the light. It is interesting to note in this connection that light has very little effect on chlorophyll formation during recovery from sulphur deficiency, the rate of synthesis being only slightly greater in the light. On the other hand, the amount of chlorophyll formed in the dark per mole of sulphate is significantly greater. This increase in yield would seem too great to be explained on the basis of more rapid decomposition in cells in the light. With respect to initiation of cell division during recovery there is a very pronounced effect of light—cell division being many times slower in the dark. It might be inferred that more sulphate is available for the processes resulting in chlorophyll formation when recovery occurs in the dark.

An analysis of the effect of various sulphur compounds on recovery could be used to advantage in studies of sulphur metabolism. While the data presented here which relate to sulphur metabolism are rather meager, brief discussion will be profitable. Since cysteine hydrochloride, dl-methionine, glutathione, thioglycollic acid and potassium thiocyanate were all found to be unavailable for recovery, it would appear that the course of sulphur metabolism does not proceed through one of these compounds. If

it does, then we must assume that the metabolism proceeds in several directions and that these reactions are not readily reversible. An alternative interpretation is impermeability of the cell to these compounds. This would not seem to be the case with cysteine since LUDWIG (9) has shown that it can be used as a source of nitrogen by this same strain of Chlorella.

All the inorganic compounds tested were available for recovery—thiosulphate, pyrosulphate, sulphite, persulphate, and hydrogen sulphide. When sulphur is supplied as H_2S the lag period is slightly longer, the acceleration in chlorophyll synthesis is slightly lower and the maximum rate of synthesis attained is less than when sulphate is supplied. This would lead to the conclusion (if questions of toxicity and rate of penetration are neglected) that sulphur in the form of H_2S must undergo more radical changes than sulphate before participation in metabolism. It is conceivable that oxidation to sulphate takes place. The response to sulphide in the dark is significantly slower than in the light. Possibly the lower oxidation potential in the cells under a condition of darkness results in slower oxidation to sulphate.

Lack of quantitative data on the course of recovery with the other inorganic compounds precludes any postulation of their relation to sulphur metabolism other than that they are assimilable.

Summary

1. Chlorella sp. was grown under controlled conditions in sulphur-deficient cultures. The development of chlorosis and the process of recovery from chlorosis have been analyzed. The chlorophyll deficit of the chlorotic cells has been put on a quantitative basis and expressed as a percentage of the normal chlorophyll content.

2. Chlorosis induced by sulphur deficiency develops in two phases due respectively to: (a) A differential between the rate of cell division and the rate of chlorophyll synthesis as they both decline to zero. This results in a decrease in the amount of chlorophyll per cell before cell division stops. (b) Slow decomposition of chlorophyll in the non-dividing cells.

3. Fat accumulates in the deficient cells (starch is the normal storage product).

4. Addition of sulphate to deficient cells results in rapid recovery from chlorosis. Although chlorophyll synthesis is evident within about 5 hours, cell division does not occur until about 24 hours after addition of sulphate. Chlorophyll formation will occur at a lower concentration of sulphate than will cell division.

5. The curve showing the formation of chlorophyll during recovery can be separated into four stages: (a) A lag period of about five hours between addition of sulphate and synthesis of chlorophyll. (b) A period of acceleration in synthesis of chlorophyll during which the rate of formation increases from zero to a maximum over a period of about five hours. (c) A period of logarithmic increase in chlorophyll at a rate faster than occurs during normal growth in full nutrient solution. (d) A period of decrease in rate of synthesis.

6. The effect of various factors on recovery was studied: light *vs.* darkness; initial degree of chlorosis; concentration of sulphate; sources of sulphur available (organic and inorganic); necessity of oxygen.

7. The chlorophyll yield during recovery is constant over the concentration range 0.01–1.93 p.p.m. of S as sulphate, about 0.34 moles of chlorophyll being formed per mole of sulphate added. It is inferred that complete absorption of sulphate takes place.

The author thanks Professor LEWIS KNUDSON for his interest in the problem and for his criticisms of the manuscript.

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LITERATURE CITED

1. EMERSON, R. Relation between maximum rate of photosynthesis and chlorophyll concentration. Jour. Gen. Physiol. **12**: 609–622. 1929.

2. ——————, and ARNOLD, W. The photochemical reaction in photosynthesis. Jour. Gen. Physiol. **16**: 191–205. 1932.

3. ——————, GREEN, L., and WEBB, J. L. Relation between quantity of chlorophyll and capacity for photosynthesis. Plant Physiol. **15**: 311–317. 1940.

4. FLEISCHER, W. The relation between chlorophyll content and rate of photosynthesis. Jour. Gen. Physiol. **18**: 573–597. 1935.

5. HOPKINS, E. F., and WANN, F. B. The effect of the H-ion concentration on the availability of iron for Chlorella sp. Jour. Gen. Physiol. **9**: 205–210. 1925.

6. ——————, and ——————. Relation of hydrogen-ion to growth of Chlorella and to the availability of iron. Bot. Gaz. **81**: 353–376. 1926.

7. ——————, and ——————. Further studies on the growth of Chlorella as affected by hydrogen-ion concentration. Bot. Gaz. **83**: 194–201. 1927.

8. KENNEDY, S. R. JR. The influence of magnesium deficiency, chlorophyll concentration, and heat treatments on the rate of photosynthesis of Chlorella. Amer. Jour. Bot. **27**: 68–73. 1940.

9. LUDWIG, C. A. The availability of different forms of nitrogen to a green alga. Amer. Jour. Bot. **25**: 448–458. 1938.

10. PEARSALL, W. H., and LOOSE, L. The growth of *Chlorella vulgaris* in pure culture. Proc. Roy. Soc. (London) Ser. B. **121**: 451–501. 1937.

11. SCHERTZ, F. M. The preparation of chlorophyll. Plant Physiol. **3**: 487–497. 1928.

12. WANN, F. B. The fixation of free nitrogen by green plants. Amer. Jour. Bot. **8**: 1–29. 1921.

STRUCTURE AND COMPOSITION OF CITRUS LEAVES AFFECTED WITH MESOPHYLL COLLAPSE[1]

F. M. TURRELL, V. P. SOKOLOFF, AND L. J. KLOTZ

(WITH SIX FIGURES)

Introduction

A pathological condition not infrequently observed in leaves of orange trees as yellow, translucent, sunken areas, has been called "mesophyll collapse" by FAWCETT (6). Neither bacteria nor fungi seem to initiate this disorder of the leaves, though they may invade the weakened and collapsed tissues secondarily. The disorder has therefore been regarded as a physiological disturbance caused by insects and by edaphic and climatic factors, or by edaphic or climatic factors alone.

FAWCETT (6) first found mesophyll collapse in the coastal districts of southern California. In a series of papers, FAWCETT et al. outlined the distribution of mesophyll collapse in South America. They found it in the coastal areas of the state of Rio de Janeiro, Brazil (11), but did not find it two hundred miles in the interior, in the state of Minas Geraes (12). It was also found fairly near the coast, in the states of Pernambuco (7) and Bahia (8), and in Sao Paulo as far as 40 miles inland and at elevations as high as 2,500 feet above sea level (8). None was noted in the Pitangueires area, some two hundred miles from the sea coast; in the state of Rio Grande do Sul, however, it was found near the coast, at Montenegro (8). It was noted at Uruguayana, somewhat inland, near the Uruguay River (8), as well as at Concordia, Argentina, also near the Uruguay River. These investigators, however, found no mesophyll collapse in any other parts of Argentina (9). In Paraguay, mesophyll collapse was found at Asuncion, near the Paraguay River (10).

The writers have made observations in North America and have noted the occurrence of mesophyll collapse in the coastal districts of the United States and Mexico. We have found it in the coastal districts of Florida, near Vero Beach, and in the coastal valleys of California. It has been observed in Arizona[2] and it probably exists, also, in some parts of Texas near the gulf. Our survey in California has shown that the disorder is present in Ventura, Santa Barbara, Los Angeles, San Bernardino, Riverside, Orange, and San Diego counties. Its general distribution in California is shown in figure 1.

This leaf condition is most readily recognized when the mature leaves are examined by transmitted light. It will then be observed that yellow, translucent areas lacking chlorophyll occur principally in the central portion of the blade, on one or both sides of the midrib (plate I, fig. 2). By reflected

light these areas on the lower surface of the leaf appear grayish and some-
what sunken below the normal leaf surface.   Necrosis or browning of the
translucent areas may also occur.   The leaves, however, are otherwise appar-
ently normal, as are the twigs on which they are carried.   So far, the symp-
toms have been observed on Valencia and Washington Navel oranges, but
not on lemons.

The disorder is of interest because it represents one of the foliar symp-
toms for which explanations have long been sought.   HAAS (14) has sug-
gested that a water deficit during the hot summer months may be involved,
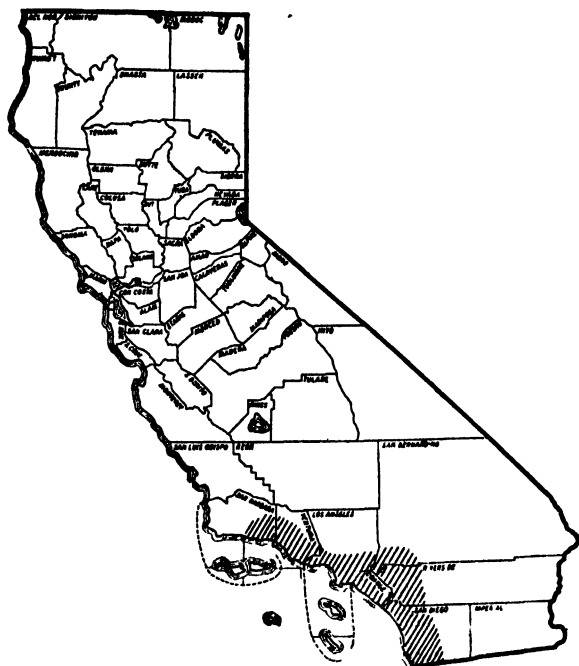
FIG. 1.   Hatched portion indicates California citrus areas in which mesophyll col-
lapse in orange leaves is prevalent.

though mesophyll collapse is seen in very young trees under conditions
where no great stress on the transpiration system has occurred.   Trees grow-
ing under optimum soil- and air-moisture conditions and subjected to occa-
sional severe water deficits, generally show the greatest incidence of meso-
phyll collapse, while trees continually subjected to water deficit are least
affected.   In groves observed by us, leaves showing the disorder were found
only on the north side of the affected tree, or they were generally distributed
around the tree.

The prevailing opinion is that the necrosis following infestation with the
citrus red mite, or red spider, *Paratetranychus citri* (McG.), is a forerunner
or an accompaniment of mesophyll collapse.   In fact, the distribution of
citrus red mite in California, as shown by QUAYLE (21), is approximately

the same as that of mesophyll collapse. Many trees not infested with citrus red mite are badly affected with mesophyll collapse, however.

The purpose of the present paper is to report studies made during 1941 and 1942 on the structure and composition of leaves of citrus trees affected with mesophyll collapse in southern California.

Materials and methods

For Ca, Mg, Na, K, Cl, and certain ash analyses, leaves were collected near Claremont, California, in early June and in late July, from Washington Navel orange trees growing on light sandy soil (Ramona loam), the pH of which, at field capacity, was 4.80 to 6.01 at the 1-foot level. A second sample of leaves for S and certain ash analyses was collected in August from the Washington Navel orange trees at Claremont and from Valencia orange trees at San Juan Capistrano. The Valencia orange trees were growing on heavy-textured Montmorillonitic yellow-clay soil (Diablo adobe clay), which had a pH of 6.15 to 6.39 at field capacity. In October, a third sample of leaves was collected from the San Juan Capistrano grove for S, P, and ash analyses.

Samples of injured and normal leaf tissue, of equal area, were excised from the leaves with a cork borer. For every sample of injured tissue, a sample of normal tissue was excised from a similar position on the blade, on the opposite side of the midrib.

Samples of Washington Navel orange leaf tissue, for sap analyses, were autoclaved 15 minutes at 15 lb. per square inch and pressed at 16,000 lb. per square inch. The sap and press cake analyses for Ca, Mg, Na, K, and Cl were made on 100 grams of sap and on 100 grams of press cake dried at 70° C., respectively. Methods of the United States Department of Agriculture Bureau of Plant Industry, Division of Irrigation Agriculture, Rubidoux Laboratory, located at Riverside, California, were used.

Analyses for total S as SO_4 were made according to the methods of the Association of Official Agricultural Chemists. Analyses for P as PO_4 were made according to methods of the Rubidoux Laboratory, using $(NH_4)_3PO_4 \cdot 12 \, MoO_3$. All leaves for these analyses were samples as previously described. The samples were heated for 2 hours at 110° C. to inactivate the enzymes and were then dehydrated at 65° C., *in vacuo*, until the material attained constant weight.

Samples of affected and healthy areas of leaves collected in August from the same groves as the material for chemical analyses, were killed in formalin acetic acid alcohol and prepared for microscopical examination by the paraffin method. Sections 12 μ in thickness were stained with Delafield's hematoxylin and safranin, and permanent mounts were made.

Results

Results of a brief study of the distribution of collapsed areas in affected leaves of Washington Navel and Valencia orange are shown in table I. For this study, each leaf was divided into two portions, the outer or marginal

portion and the inner or middle portion; the collapsed areas at proximal, central, and distal locations in these portions were counted. It was clear that the incidence of the disorder occurred principally in the central portion of the leaves.

Of the Washington Navel orange leaves which exhibited mesophyll collapse (table I), 17.2 per cent. were small, 54.4 per cent. were medium, and 28.4 per cent. were large. Of the Valencia orange leaves which exhibited mesophyll collapse, 31.7 per cent. were small, 40.0 per cent. were medium, and 28.3 per cent. were large. This is what would be expected from the normal size distribution of orange leaves. In the Washington Navel orange leaves, 68.7 per cent. of the translucent areas contained necrotic areas (brown spots); in the Valencia orange leaves, 40.3 per cent.

TABLE I

DISTRIBUTION OF AREAS AFFECTED WITH MESOPHYLL COLLAPSE IN ORANGE LEAVES, EXPRESSED IN PERCENTAGE OF TOTAL NUMBER OF COLLAPSED AREAS

LOCATION	WASHINGTON NAVEL		VALENCIA	
	MARGINAL LEAF PORTION	MIDDLE LEAF PORTION	MARGINAL LEAF PORTION	MIDDLE LEAF PORTION
	%	%	%	%
Proximal	4.0	8.0	9.3	16.3
Central	18.7	42.0	13.9	33.4
Distal	9.3	18.0	10.8	16.3

From a comparative histological examination of normal green leaf tissue and of affected leaf tissue, it was evident that the injured tissue contained collapsed spongy mesophyll cells (plate I, figs. 3–6). Until the affected areas dried, the palisade tissue was apparently normal (plate I, figs, 3–4). Intercellular space was reduced in the abnormal tissue, owing to the collapse of much of the spongy mesophyll and the enlargement of certain spongy mesophyll cells which had not collapsed. The diameters of the enlarged cells averaged 35 μ, whereas the diameters of normal cells averaged 18 μ. The walls of the enlarged cells had a maximum thickness of 2 μ; walls of normal sponge cells had a maximum thickness of 0.8 μ. Collapsed and injured tissue stained deeply with safranin, as shown by the dark regions in figure 4. The disorder was not confined to one vein islet, but included several to many.

Only rarely was the palisade tissue abnormal above the abnormal spongy mesophyll cells. As shown in plate I, figure 6, the palisade cells are shortened and not elongated. This suggests that, in addition to hypertrophy and collapse in the sponge tissue, on rare occasion hyperplasia may be observed in the palisade tissue, a condition reminiscent of boron deficiency. The palisade mesophyll directly above the affected spongy mesophyll is generally normal, however. This is confirmed by examination of tangential sections through palisade tissue above normal spongy mesophyll and above abnormal spongy mesophyll.
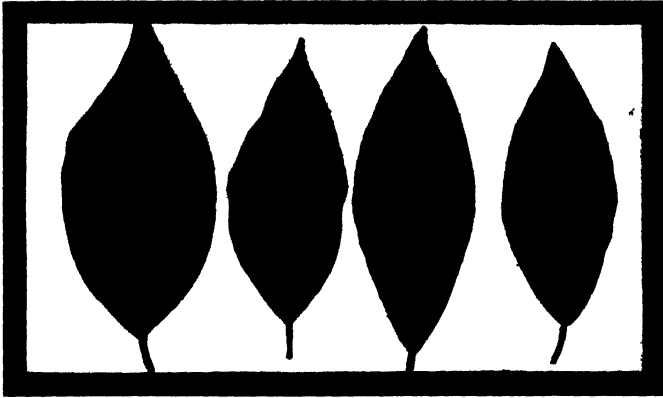
FIG. 2. Mesophyll collapse of sweet orange leaves photographed by transmitted light (From color plate 27, Klotz and Fawcett, [19])
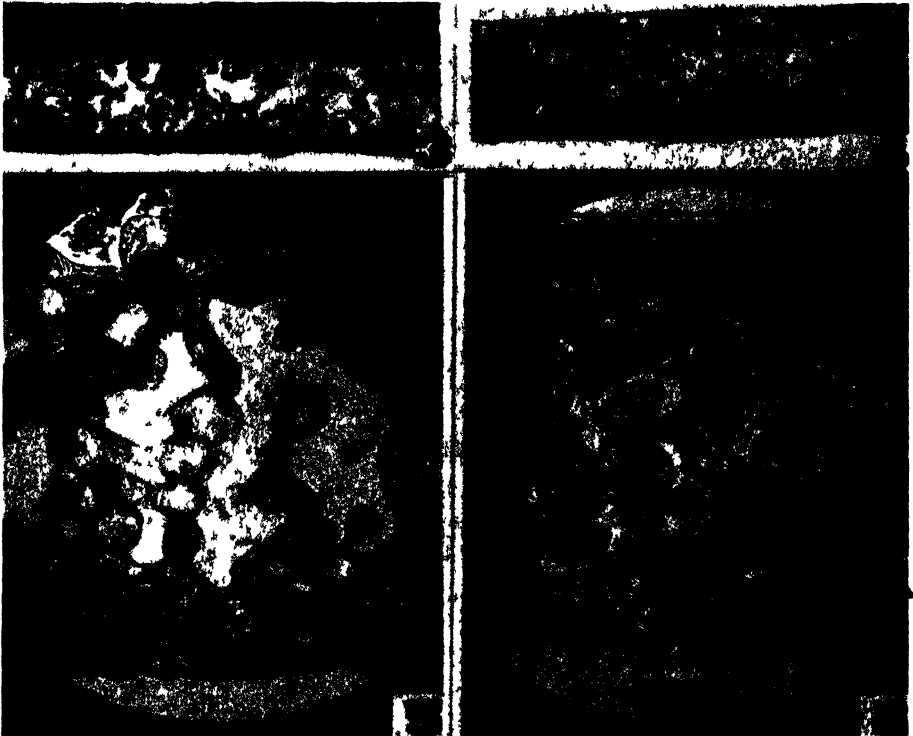
Photomicrographs of transverse sections of Washington Navel orange leaves.
FIG. 3. Section through normal portion of leaf showing greater leaf thickness and large intercellular space   x 64
FIC 4. Section through abnormal portion of leaf showing reduced leaf thickness, collapsed mesophyll and abnormally enlarged mesophyll cells. x 64
FIG. 5. Same section as Fig. 3.   x 287.
FIG 6. Same section as Fig. 4.   x 287.

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TABLE II

SULPHATE AND CARBONATE ASH IN COLLAPSED AND NORMAL LEAF TISSUE OF WASHINGTON
NAVEL AND VALENCIA ORANGE, EXPRESSED IN GRAMS PER 100 GRAMS
OF DRY SUBSTANCE

SAMPLE	LEAF TISSUE	SO_4 ASH	CO_3 ASH
WASHINGTON NAVEL			
		gm.	*gm.*
1	Collapsed	14.8	11.1
1c	Normal	16.8	11.4
2	Collapsed	17.8	11.2
2c	Normal	18.3	11.5
3	Collapsed	17.7	11.6
3c	Normal	18.9	11.2
4	Collapsed	18.4	11.3
4c	Normal	19.6	11.2
VALENCIA			
7	Collapsed	15.8	8.9
7c	Normal	17.8	13.5

The normal spongy mesophyll, as seen in tangential section, contains intercellular space. The abnormal spongy mesophyll, prior to collapse, shows hypertrophy of certain sponge cells so abutting that no intercellular space is visible.

While normal leaves vary greatly in thickness, sections of the same leaf at loci where normal tissue is found, and where mesophyll collapse is apparent, differ greatly. The averages of numerous measurements indicated that, in normal tissue, the leaf thickness was 283 μ, palisade thickness was 91 μ, and sponge thickness was 183 μ; while in abnormal tissue, the leaf thickness was 255 μ, palisade thickness was 87 μ, and sponge thickness was 153 μ. It was of especial interest to note that the cuticle was intact in all sections. Chloroplasts seemed to be fewer in number in the enlarged spongy mesophyll cells than in the normal.

TABLE III

DISTRIBUTION OF ALKALINE-EARTH AND ALKALI BASES AND OF CHLORIDE BETWEEN EXPRESSED
SAP AND PRESS CAKE OF COLLAPSED AND OF NORMAL LEAF TISSUE OF WASHINGTON
NAVEL ORANGE, EXPRESSED AS MILLIEQUIVALENTS PER 100 GRAMS OF
SAP OR DRY SUBSTANCE, RESPECTIVELY

SAMPLE	LEAF TISSUE	EXPRESSED SAP					PRESS CAKE				
		Ca	Mg	K	Na	Cl	Ca	Mg	K	Na	Cl
		m. eq.	*m. eq.*	*m.eq.*	*m.eq.*	*m.eq.*	*m.eq.*	*m.eq.*	*m.eq.*	*m.eq.*	*m.eq.*
3	Collapsed	44	4	12	3	6	234	29	13	14	1
3c	Normal	68	1	10	1	3	256	11	8	5	1
4	Collapsed	44	3	7	3	3	236	22	8	6	1
4c	Normal	91	2	5	1	1	278	14	7	3	2

Chemical analyses indicate a number of differences between collapsed and normal tissue. As shown in table II, the sulphate ash in the dry matter of collapsed tissue was in every case lower than that in the dry matter of normal tissue. On the other hand, the carbonate ash was about the same in both normal and collapsed tissue.

The Ca content of the sap of collapsed tissue was lower than that of the sap of normal tissue (table III) but the Mg, K, Na, and Cl contents were higher in the sap of collapsed tissue than in that of normal tissue (table III).

The Ca content of the press cake of collapsed tissue, also, was lower than that of normal tissue (table III); and the Mg, K, and Na contents were higher in the press cake of collapsed tissue than in that of normal tissue (table III). There was no significant difference in the Cl content of the press cake from normal and collapsed tissue (table III).

TABLE IV

ALKALINE-EARTH AND ALKALI BASES IN COLLAPSED AND IN NORMAL LEAF TISSUE OF WASHINGTON NAVEL ORANGE, EXPRESSED IN MILLIEQUIVALENTS PER 100 GRAMS OF DRY SUBSTANCE

SAMPLE	LEAF TISSUE	Ca	Mg	K	Na	TOTAL Ca, Mg, K, AND Na
		m. eq.	m. eq.	m. eq.	m. eq.	m eq.
1	Collapsed	177	19	63	5	264
1c	Normal	229	11	32	1	273
2	Collapsed	230	22	40	3	295
2c	Normal	258	13	23	3	297
3	Collapsed	245	30	15	15	305
3c	Normal	297	12	14	5	328
4	Collapsed	277	25	14	9	325
4c	Normal	382	15	13	4	414

Analyses of unexpressed normal and collapsed leaf tissues for alkaline-earth and alkali bases are shown in table IV. The analyses of samples 1, 1c, 2, and 2c are averages of three separate examinations; namely, analysis of plant ash, the sum of 30 per cent. C_2H_5OH-soluble and -insoluble fractions, and the sum of fractions soluble and insoluble in 0.05 N $BaCl_2$ in 30 per cent. C_2H_5OH. Analyses of samples 3, 3c, 4, and 4c are summations of analyses of expressed sap and of press cake. The results shown in table IV are in agreement with the direction of magnitudes of the cation contents in the sap and press cake analyses shown in table III; that is, the Ca is lower in the collapsed tissue than in the normal tissue, and the other cations are higher. The Na content of sample 2 presents the only exception: it was the same as that of the normal tissue of sample 2c.

Analyses made of the unexpressed normal and collapsed leaf tissues for total S as SO_4 and P as PO_4 are shown in table V. In samples 5 and 5c from Washington Navel orange leaves, and 6 and 6c from Valencia orange leaves, the normal tissue contained more S than the collapsed tissue. In the Va-

lencia leaves of samples 7, 7c, 8, and 8c, however, the' S content of the collapsed tissue was higher than that of the normal tissue. In all cases, the P values were higher in the collapsed tissue than in the normal tissue. Semiquantitative microanalyses in samples 5, 5c, 6, and 6c indicated larger concentrations of P in collapsed tissue. The percentage of moisture in collapsed and normal samples of leaf tissue are also shown in table V. The normal leaf tissue of Washington Navel orange and of Valencia orange contained a higher percentage of moisture than the collapsed tissue. The pH of normal leaf sap was 5.42, which was slightly, but not significantly, higher than the pH of sap from collapsed leaf tissue, which was 5.37.

TABLE V

PERCENTAGE MOISTURE, TOTAL SULPHUR, AND TOTAL PHOSPHORUS IN COLLAPSED AND NORMAL LEAF TISSUE OF WASHINGTON NAVEL AND VALENCIA ORANGE, EXPRESSED IN MILLIGRAM ATOMS PER 100 GRAMS OF DRY SUBSTANCE

SAMPLE	LEAF TISSUE	MOISTURE	S	P
WASHINGTON NAVEL				
		mg. atom.	mg. atom.	mg. atom.
5	Collapsed	47.10	7.3*	3.8
5c	Normal	48.16	8.3*	3.0
VALENCIA				
6	Collapsed	41.87	9.2*	3.5
6c	Normal	42.83	12.1*	3.1
7	Collapsed		23.5	2.9
7c	Normal		20.5	2.3
8	Collapsed		28.2	2.8
8c	Normal		23.6	1.7

* The authors wish to thank PROFESSOR A. J. HAAGEN-SMIT, California Institute of Technology, for the microanalyses reported here.

Comparison of our leaf analyses with those of other workers dealing with various nutrient deficiencies and excesses, indicated that the principal differences between their results and ours lay in the high Mg values obtained by us. Consideration of the soil types where mesophyll collapse occurred in California, suggested that Mg^{++} might be associated with the disorder.

Several experiments were run with potted sour-orange seedlings, which were watered with 50 e.p.m. of $MgSO_4$ solution for two weeks and then leached with distilled water. After two days of the latter treatment, leaf symptoms of mesophyll collapse developed. The leaves were sectioned and examined. Similarity to mesophyll collapse was evident.

Discussion

Ignited residue weighed as carbonate ash is obtained at the relatively low temperatures of dull-red heat. It contains, chiefly, combinations of carbonates, oxides, and phosphates of the alkaline-earth and alkali bases; small amounts of Si, SO_4, and Cl; and other quantitatively minor constituents.

Organic compounds of the bases, such as $CaC_2O_4 \cdot H_2O$, for example, and nitrates are converted into carbonates or oxides in the course of the combustion.

Sulphate ash, on the other hand, is obtained at much higher temperatures. The plant material, prior to the combustion, is treated with H_2SO_4. Under such conditions the ignited residue consists largely of sulphates and phosphates. As the equivalent combining weight of SO_4 is higher than that of CO_3 or O, correspondingly higher figures for the SO_4 ash are obtained, all other things being equal.

In view of these considerations, it is possible to interpret the results shown in table II in the following manner. Organic compounds of the bases are less prominent in the collapsed tissue than in the normal tissue, as evidenced by the consistently smaller differences between the SO_4 and the CO_3 ash of the collapsed tissue. This conclusion is also supported by the fact that SO_4 ash is higher in the normal tissue than in the collapsed tissue, while carbonate ash, in all samples except those of Valencia orange, is nearly identical in the two tissues. Furthermore, were the CO_3 ash residues largely identical in kind, the trends in the SO_4 ash would not have become manifest. One may infer, therefore, that, while some quantitatively important "acidic" constituents of the CO_3 ash of collapsed tissue are similar in their equivalent combining weight to those of the CO_3 ash of normal tissue, they are not converted to SO_4 in the course of the SO_4 ash determination. Tertiary PO_4 would be the most likely type of substance responsible for this difference. If it is assumed that the observed difference is due to tertiary PO_4, the collapsed tissue would be expected to contain more PO_4 than the normal tissue, but less of the organic compounds of the alkaline-earth and alkali bases. Actually, analyses of the tissue for PO_4 showed that this was the case.

Relative amounts of Ca in the sap phase of collapsed tissue are much lower than in the corresponding samples from normal tissue. The ratio of Ca to the alkali bases, for example, is 3 and 4 in the sap of collapsed tissue as against 6 and 15 in that of normal tissue, for samples 3 and 4 and 3c and 4c, respectively. In the press cake analyses, similar trends are also manifest; but, recognizing that variable amounts of sap are retained in the press cake against a pressure of 16,000 lb. per square inch, we have not relied on the press cake analyses alone.

Hydration of colloids contained in the leaf tissue is most probably influenced by the kind of cations combined or associated with the colloids. It is reasonable to infer that, all other things being equal, any increase in the proportion of monovalent cations, that is, K^+ and Na^+, in the liquid phase in equilibrium with the colloidal surfaces, would produce corresponding changes in the cations adsorbed by the colloids; whereupon the extent of hydration of the latter would in all probability be increased. In fact, STUEWER (23) showed that, in aqueous solution, pectic acid is 21 per cent. hydrated; pectin, 25; calcium pectate, 35; and sodium pectate, 38. Such an increase in hydration would also be brought about by the removal of Ca^{++} by replacement with monovalent cation.

Precipitation or translocation of Ca^{++} would be expected after replacement, and our analyses suggest the latter possibility rather than the former. Any increase in amounts of cations other than Ca^{++}, would tend to increase the mobility of Ca^{++} in the plant tissue, whether this Ca is in the adsorbed or in the precipitated form.

STUEWER (**23**), as a result of his experiments, states that the sodium salts of pectin are highly dissociated, that the salts of divalent metals are considerably less dissociated, and that the acid is still less dissociated. These results are in agreement with the reported facts that sodium salts hinder jelling, while calcium chloride promotes it. In our own studies, despite variations in the absolute amounts of the bases and in every comparison of collapsed tissue with corresponding normal tissue, Mg, K, and Na were always higher in the collapsed tissue than in the normal tissue, while Ca was always lower. This suggests that the weakening of the gel structure in the spongy mesophyll cells may be caused by the high concentrations of Mg^{++}, K^+, and Na^+, and the low concentrations of Ca^{++}, so that in irregularly occurring periods of unusual transpirational stress, collapse of the cells results.

The work of KERTESZ et al. (**18**) seems to support this interpretation of results. They found that Ca salts added to canned tomatoes result in the better retention of the original firmness of the fruit. It was postulated by KERTESZ (**17**) that the addition of Na or K salt accomplished just the opposite of the Ca effect, inasmuch as Ca is removed from the tissue by an exchange of ions. This reaction was confirmed by GREENLEAF, according to KERTESZ et al. (**18**). GREENLEAF (**13**) has further shown that the Ca salt improves the texture of the canned product by forming calcium pectate.

The findings of STEWARD, STOUT, and PRESTON (**22**), in their respiration studies, indicate that K^+ increases the mobility of pectin. They found that KBr at high oxygen tension, through the action of K^+ on Ca^{++}, caused the transfer of a uronic acid from potato disks to blotting papers; this, when calculated as pectin, accounted for the concomitant loss of carbon on their balance sheets. It also seems possible that such colloids as proteins might act in a similar manner under the influence of K^+ or Na^+.

The fact that normal leaf tissue has a higher percentage of moisture than tissue containing collapsed spongy mesophyll cells, may be interpreted as support of the explanation of the mechanism of collapse, since weakening of the gel structure to the extent where collapse would occur would be followed by a loss in water and necrosis. This process is commonly observed as a result of freeze injury in citrus fruits and affects entire vesicles.

While these facts suggest the mechanism of the collapse of the spongy mesophyll, they do not contain any suggestion as to the mechanism of the enlargement of certain of the spongy mesophyll cells found among the collapsed cells.

The theory that the citrus red mite, or red spider, may be responsible for mesophyll collapse seems to offer an explanation of the enlarged cells, since the effect of certain indole derivatives and excreta from insects is known to

cause intumescences in leaves (20). Mesophyll collapse may be abundant, however, where red spider infestations are light; and in greenhouse studies with red spider, we failed to produce symptoms of mesophyll collapse. Macroscopic examination of the surface of certain leaves from the field, exhibiting mesophyll collapse, failed to show symptoms of red spider injury, such as silvering of the upper surface of the leaf. Microscopic examination in cross section failed to show spots in the palisade parenchyma lacking chlorophyll, which we have found in silvered leaves having heavy infestations of red spider. We have therefore questioned the red spider explanation of mesophyll collapse, despite the similar distribution of the two.

HAAS and THOMAS (15) have shown that in leaves from Lisbon lemon trees, severe injury is correlated with a high S content ranging from 0.68 to 1.10 per cent. of the dry matter. In the field, SO_4 injury occurred where $NaNO_3$ was absent in fertilizers containing blood and bone meal and K_2SO_4; or blood, bone meal, and K_2SO_4; or impure $CaH_4(PO_4)_2$ (superphosphate), alone or in combination with organic fertilizers, but without $NaNO_3$. The SO_4 content of mesophyll-collapsed tissue, however, is about normal. Values agree well with those reported by KELLEY and CUMMINS (16) for normal leaves and with those reported by HAAS and THOMAS (15) for uninjured leaves. But our values are much lower than those reported by HAAS and THOMAS for leaves showing severe SO_4 injury.

Analyses of CHAPMAN and BROWN (2) on leaves of P-deficient navel-orange trees, show a disturbed ionic balance in the leaf tissue similar to that found by the authors in mesophyll collapse, except that the P content was low in their material and high in ours. The values of CHAPMAN and BROWN (3) for S-deficient plants, as compared with healthy plants, agree with our values for collapsed tissue of mature leaves, as compared with normal leaf tissue, except in S content, where our results were variable. The values of CHAPMAN and LIEBIG (4) for high-SO_4 plants at various nitrogen levels are similar to our results for Ca, K, and P; but Mg is low and S is high for high-SO_4, while in mesophyll-collapsed tissue, Mg is high and S is variable. Only a few points of agreement were noted between their chloride series and our results.

EATON (5) grew eight crops in nutrient media containing 50 and 150 milliequivalents of Cl and 50, 150, and 250 milliequivalents of SO_4 per liter. His sap analyses of these crops for Ca, Mg, K, and Na, as well as the sum of the cations in several instances, show the same trend for high SO_4 plants, as compared with control, as our results show for collapsed tissue as compared with normal tissue.

From the preceding comparisons, it may be surmised that mesophyll collapse is related chemically to an unbalance in the ionic constituents of the plant. It is possible that this unbalance may be due to excesses or deficiencies in soil constituents.

Morphologically, the appearance of mesophyll collapse may resemble the external leaf symptoms sometimes obtained in water culture in B deficiency

or in cultures at high nitrate levels, using $Ca(NO_3)_2$. 'Certain leaves showing symptoms of mesophyll collapse were obtained from such cultures[3] for histological examination. None of these leaves contained collapsed spongy mesophyll cells, but they showed distinct histological characteristics peculiar to the two types of cultures from which they were obtained. CAMP[4] states that he has produced symptoms of mesophyll collapse by treating dry citrus trees with excesses of KNO_3, or with $Ca(NO_3)_2$. BAIN and CHAPMAN (1) produced similar symptoms in grapefruit cultures, using $Ca(NO_3)_2$ in combination with waterlogged soil.

Further work will be necessary to determine whether one or several types of ionic unbalance may bring about typical external and internal symptoms of mesophyll collapse.

Summary

Mesophyll collapse apparently occurs in leaves of orange trees in the coastal regions of all southern California orange districts. It is confined largely to leaves of orange and has seldom been seen on those of lemon. Leaves exhibiting mesophyll collapse have chlorotic, translucent, water-soaked-appearing areas, principally in the central portion of the leaf blade, although collapse symptoms may occur anywhere in the lamina; brown necrotic spots may develop in the translucent areas. Histological examination of affected leaf tissue shows enlarged sponge cells interspersed with collapsed sponge cells, reduced intercellular spaces, and chlorophyll-depleted spongy mesophyll cells.

Ca was invariably found to be lower in collapsed tissue than in normal tissue, while K, Mg, Na, Cl, and P were somewhat higher than in normal tissue. The ratio of Ca to K was accordingly lower in the collapsed tissue than in the normal tissue. Distribution of the alkaline-earth and alkali bases between the liquid (sap) and the solid phases of autoclaved leaf tissue likewise showed similar differences in absolute and in relative amounts of Ca and K. No consistent difference in the S content of collapsed and normal tissue was found. Consistently lower percentages of SO_4 ash were found in the collapsed tissue than in the normal tissue, while the CO_3 ash was the same in both normal and collapsed tissue.

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LITERATURE CITED

1. BAIN, F. M., and CHAPMAN, H. D.   Nitrate fertilizer additions to waterlogged soils in relation to oxygen deficiency.   Soil Sci. **50**: 357–367. 1940.

[3] Through the courtesy of DR. A. R. C. HAAS.

[4] CAMP, DR. A. F.   In a letter to PROF. H. S. FAWCETT, dated January 27, 1943.

2. CHAPMAN, H. D., and BROWN, S. M. The effects of phosphorus deficiency on citrus. Hilgardia **14**: 161–181. 1941.

3. —————, and —————. The effects of sulfur deficiency on citrus. Hilgardia **14**: 183–201. 1941.

4. —————, and LIEBIG, G. F., JR. Nitrate concentration and ion balance in relation to citrus nutrition. Hilgardia **13**: 141–173. 1940.

5. EATON, FRANK M. Toxicity and accumulation of chloride and sulfate salts in plants. Jour. Agr. Res. **64**: 357–399. 1942.

6. FAWCETT, H. S. Citrus diseases and their control. 2nd ed. McGraw-Hill Book Company. New York. 1936.

7. —————, and BITANCOURT, A. A. As doenças dos citrus no Estado de Pernambuco. Boletim da Secretaria de Agricultura, Industria e Comercio (Pernambuco, Brasil) **2**: 317–326. 1937.

8. —————, and —————. Relatorio sobre as doenças dos citrus nos Estados de Pernambuco, Bahia, São Paulo e Rio Grande do Sul. Rodriguesia **3**: 213–236. 1937.

9. —————, and —————. Observaciones sobre las enfermedades de los citrus en el Uruguay. Revista de la Associación do Ingenieros Agronomos (Montevidea, Uruguay) **12**: 3–8. 1940.

10. —————, and —————. Observações sobre as doenças dos citrus no Paraguay. Biologico **6**: 289–296. 1940.

11. —————, GRILLO, H. V. S., and BITANCOURT, A. A. Relatorio sobre as doenças dos citrus no Districto Federal, Estado do Rio de Janeiro e Minas Geraes. I. Districto Federal e Estudo do Rio de Janeiro. Rodriguesia **2**: 329–340. 1936.

12. —————, and MÜLLER, A. S. Relatorio sobre as doenças dos citrus no Districto Federal, Estado do Rio de Janeiro e Minas Geraes. II. Estado de Minas Geraes. Rodriguesia **2**: 340–344. 1936.

13. GREENLEAF, C. A. Suggestions on use of calcium chloride in canned tomatoes. Canner **91**: 11–12. 1940.

14. HAAS, A. R. C. Mesophyll collapse of citrus leaves. California Citrog. **22**: 114. 1937.

15. —————, and THOMAS, E. E. Effect of sulfate on lemon leaves. Bot. Gaz. **86**: 345–354. 1928.

16. KELLEY, W. P., and CUMMINS, A. B. Composition of normal and mottled citrus leaves. Jour. Agr. Res. **20**: 161–191. 1920.

17. KERTESZ, Z. I. The effect of calcium on plant tissues. Canner **88**: 26–27. 1939.

18. —————, TOLMAN, T. G., LOCONTI, J. D., and RUYLE, E. H. The use of calcium in the commercial canning of whole tomatoes. New York Agr. Exp. Sta. Tech. Bull. 252. 1940.

19. KLOTZ, L. J., and FAWCETT, H. S. Color handbook of citrus diseases. University of California Press, Berkeley and Los Angeles. 1941.

20. LaRue, Carl D.   The rôle of auxin in the development of intumescences on poplar leaves; in the production of cell outgrowths in the tunnels of leaf-miners; and in the leaf-fall in Coleus.   Amer. Jour. Bot. (Abstract) 22: 908.   1935.

21. Quayle, Henry J.   Insects of citrus and other subtropical fruits. Comstock Pub. Co.   Ithaca, New York.   1941.

22. Steward, F. C., Stout, P. R., and Preston, C.   The balance sheet of metabolites for potato discs showing the effect of salts and dissolved oxygen on metabolism at 23° C.   Plant Physiol. 15: 409–447. 1940.

23. Stuewer, Reinhold F.   The hydration and physicochemical properties of pectin and its derivatives.   Jour. Phys. Chem. 42: 305–315. 1938.

RADIANT ENERGY NOMENCLATURE[1]

Robert B. Withrow

Introduction

This report is presented in an endeavor to promote among plant physiologists a more consistent and uniform usage of nomenclature pertaining to radiant energy. The committee has tried to assemble the most authoritative and useful terminology recommendations in a readily available form. No endeavor has been made, however, to present a complete discourse on radiant energy nomenclature since it is considered better to avoid the introduction of terms and relationships which are not likely to be used by plant physiologists.

The nomenclature committees of the Optical Society of America and of the Illuminating Engineering Society have been instrumental in bringing about the standardization of radiant energy terminology (3, 4, 5, 6, 7, 8, 9, 10, 11). The present paper is based upon the latest official reports of these two societies (5, 6, 8). Definitions of terms not covered in these reports have been taken from other authoritative sources (1, 2, 12, 13).

The Physical Methods Committee has found it difficult to decide which terms would be the more useful for plant physiologists where there is disagreement between the terminology recommended by the Optical Society and that recommended by the Illuminating Engineering Society. It finally was decided to give preference to the terms recommended by the Optical Society but to include in parentheses the equivalent terms of the Illuminating Engineering Society. It should be noted that the last report of the Illuminating Engineering Society Committee (5) has been accepted by the American Standards Association and probably will be followed rather closely in the engineering fields. The latest published report of the Optical Society (8) is preliminary in nature but a final report which includes certain revisions is being prepared for publication. The Physical Methods Committee was informed of the revisions to be made in terms to be included in the present report and these revisions have been taken into account in the preparation of this paper.

Radiometric and photometric terms

Table I presents the recommended system of nomenclature for radiometric and photometric terms. It will be noted that for each radiometric term, there is a corresponding photometric term. *Photometry* is considered as a special case of *radiometry* in which the radiometer or detector of radiant energy is the human eye or a selective detector employed as a substitute for the eye. The plant physiologist seldom is justified in using photometric

[1] A report of the Physical Methods Committee of the American Society of Plant Physiologists. Membership: R. B. Withrow, Chairman, S. T. Dexter, K. C. Hamner, and B. S. Meyer.

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TABLE I

RADIOMETRIC AND PHOTOMETRIC NOMENCLATURE AND UNITS

| | RADIOMETRIC | | | PHOTOMETRIC | |
TERM*	SYMBOL AND DEFINING EQUATION	UNITS	TERM	SYMBOL AND DEFINING EQUATION	UNITS
Radiation			Lumination (Illumination)		
Radiator, source of radiant energy			Luminator (Lamp, Illuminant)		
Radiant energy	U	erg, joule, calorie	Luminous energy (Light)	$Q = Fdt$	lumerg, lumen hour
Spectral radiant energy	$U_\lambda = dU/d\lambda$				
Radiant density	$u = dU/dv$	erg/cm³	Luminous density	$q = dQ/dv$	lumerg/cm³
Radiant flux	$P = dU/dt$	erg/sec, watt	Luminous flux	$F = dQ/dt$	lumen, lumerg/sec
Radiant emittance (Radiancy)	$W = dP/da$	watt/cm²	Luminous emittance	$L = dF/da$	lumen/cm³
Radiant intensity	$J = dP/d\omega$	watt/steradian	Luminous intensity (Candlepower)	$I = dF/d\omega$	candle
Radiance (Steradiancy)	$N = \dfrac{dP}{d\omega(da\cos\theta)}$	$\dfrac{\text{watt/steradian}}{\text{cm}^2}$	Luminance (Brightness)	$B = \dfrac{dF}{d\omega(da\cos\theta)}$	candle/cm², stilb
Irradiation			Illumination		
Irradiance (Irradiancy)	$H = dP/da$	watt/cm²	Illuminance (Illumination)	$E = dF/da$	footcandle, lux, phot
Zero level radiant energy	$U_{\lambda_1}^{\lambda_2} = 0$		Dark, zero level luminous energy	$Q = 0$	
Radiometry			Photometry		
Spectroradiometry			Spectrophotometry		

* Where there is disagreement between the terms of the Optical Society of America and those of the Illuminating Engineering Society, the O.S.A. terms are given first and the I.E.S. terms follow in parentheses.

terms in dealing with physiological problems since the radiant energy is used in most instances to irradiate plants or non-living physical or chemical systems. Under these circumstances, the radiant energy is not being used for visual purposes. Photometric terms may be used logically in cases, however, where the eye is involved as the detector of the radiant energy, as in the use of visual optical instruments in general and in specifying conditions concerning the visual inspection of objects. As will be discussed later, photometric units may be used as units of irradiance under those conditions where the eye or another detector of similar spectral sensitivity is used as the selective radiometer.

Photo- has been used extensively as a prefix in the restricted sense to mean light and in the more general sense as synonymous with radiant energy. Common uses with the latter connotation are photograph, photochemistry, and photon. Many such terms as these have become so well established that there is little value in attempting to change their prefix to the more logical one of *radi-*.

Radiant energy is that form of energy which is propagated through space in the form of electromagnetic waves. It may be specified in terms of the

TABLE II

CONVERSION FACTORS FOR ENERGY UNITS

UNITS	ERG	JOULE	GRAM-CALORIE
Erg	1.	10^{-7}	0.239×10^{-7}
Joule	10^7	1.	0.239
Gram-calorie	4.18×10^7	4.18	1.

spectral region involved such as visible radiant energy, ultraviolet radiant energy, or X-radiant energy (X-rays). It also may be specified in terms of the source of the energy such as incandescent-lamp radiant energy or solar radiant energy. It is preferable to use the term solar radiant energy rather than the commonly used terms sunlight and daylight when dealing with plant physiological problems.

The units of radiant energy are the same as those for other forms of energy. Some of the most frequently used units are the erg, joule, and gram-calorie. The quantitative relationship between these units is presented in table II.

The photometric correlate of radiant energy is *luminous energy* or *light* which is radiant energy evaluated with respect to its capacity to produce visual sensation. Light is, therefore, radiant energy spectrally evaluated in accordance with the luminosity curve of the human eye. Radiant energy in the region of 555 mμ, which appears yellow-green to the eye, is more efficient in producing the sensation of brightness than that of any other region of the spectrum. Longer or shorter wavelengths are less efficient. The standard luminosity curve drops to about one-tenth of one per cent. of the maximum value at 410 mμ in the blue-violet and 720 mμ in the red. Radiant energy of wavelengths outside of these limits in the ultraviolet and infrared,

respectively, logically cannot be termed light because it has little or no capacity to produce direct visual sensation. Therefore, such terms as "ultra-violet light" and "infrared light" are misnomers. Within the limits of the visible spectrum, radiant energy may be termed light *only* if it is being evaluated in terms of its capacity to produce visual sensations.

Radiation is the process by which radiant energy is generated and emitted by a source, and propagated through space. Radiation is not synonymous with radiant energy although it frequently has been used in that sense. *Lumination (illumination)* is the photometric analog of radiation and is the term applied to the generation and propagation of luminous energy or light.

A *radiator* is any source of radiant energy, regardless of the region of the spectrum involved. The term is usually applied only to that part of the mechanism which actually is radiating, such as the filament of an incandescent lamp or the target of an X-ray tube.

A *luminator (lamp, illuminant)* is any artificial source of *luminous energy (light)*. The term *lamp*, however, is frequently applied to sources of near ultraviolet, visible, and near infrared radiant energy. The term usually includes not only the radiator but the whole permanently attached assembly such as the electrode supports, enclosure, and mounting base.

Special attention is directed to the common but very confusing practice of referring to lamps by trade names. Such terms as Mazda, Osram, Pointolite, Uviarc, Cooper-Hewitt and others are trade names which frequently have little significance to the reader as to the nature of the lamp employed. It is preferable to designate lamps by more descriptive technical terms as incandescent, high pressure mercury arc, sodium arc, or as the case may require. Additional informative data as wattage and color temperature should be included where it is necessary for proper interpretation of the experimental results.

Radiant density is the volume density of radiant energy and expresses the amount of the radiant energy existing within a unit volume of the space under consideration. The unit is the erg per cubic centimeter. *Luminous density* is the analogous photometric term.

Every useful source of energy is evaluated in some unit of power which is a measure of the time rate of flow of the energy. In the case of a source of electrical energy such as a generator, the unit of power is the joule per second or watt. Similarly, sources of radiant energy may be evaluated in terms of the time rate of flow of the radiant energy into the surrounding space which is termed the *radiant flux* of the source. The term radiant flux is not limited to sources alone but is generally applicable to any system involving the transfer of radiant energy. As with any other power term it has nothing to do with the direction of flow of the energy or the dimensional configuration of the system. It may be applied with equal logic to receivers of radiant energy as well as to generators.

Luminous flux is the time rate of flow of luminous energy (light) and is expressed in terms of the *lumen*. The *candle* is the unit of luminous in-

tensity. The unit used in the United States is a specified fraction of the average horizontal candlepower of a group of 45 carbon-filament lamps, preserved at the National Bureau of Standards, when the lamps are operated at specified voltages. This unit is identical, within the limits of uncertainty of measurement, with the international candle established in 1909.

It is evident that the complete surface of a sphere having a theoretically uniform point source of luminous intensity of one candle at its center would intercept a total flux of 4π lumens. Since the area of a sphere is $4\pi r^2$, any portion of the total surface equivalent in area to the square of the radius will intercept a luminous flux of one lumen. The solid angle thus formed, having its apex at the source and its base enclosing a surface on the sphere equivalent in area to the square of the radius, is called a *steradian*. The lumen is therefore a unit of luminous flux and is defined as the luminous flux passing through a unit solid angle, or steradian, from a uniform point source having a luminous intensity of one candle. It is also the flux on a unit surface, all points of which are at unit distances from a uniform point source of one candle.

It frequently is desirable to specify geometrically the radiant flux of the source. Three intensity terms and their photometric analogs have been developed for this purpose. These terms serve to specify the radiant flux emitted by the source as a function of various geometrical units. *Radiant emittance (radiancy)* is an expression of the radiant flux emitted per unit area of the source. This term usually is applied to distributed sources of large area such as the fluorescent lamp. The unit is the watt per square centimeter. *Luminous emittance* is the corresponding photometric term.

When sources such as a carbon arc or a projection lamp are employed in optical systems which are large as compared with the dimensions of the radiator, the source may, for all practical purposes, be considered as a point source having no surface area. Therefore, the quantity that is of interest is not the flux emitted per unit area but the radiant flux emitted per unit solid angle, or steradian, which is termed the *radiant intensity*. The unit is the watt per steradian. *Luminous intensity (candlepower)* is evaluated in terms of the candle or of the lumen per steradian.

Radiance (steradiancy) is a term which is applicable to diffuse sources. It is a combination of the two previous terms and may be defined as the radiant flux per unit solid angle per unit of projected area and is expressed as the watt per steradian per square centimeter. The photometric term is *luminance (brightness)* and is expressed in the unit of the candle per square centimeter of projected area, or *stilb*.

The terms *radiant emittance (radiancy)*, *radiant intensity*, and *radiance (steradiancy)* and their photometric analogs are intensity terms which are extensively used in papers in engineering and physics dealing with radiant flux measurements on sources of radiant energy. Their primary use to physiologists is in making calculations concerning the irradiances obtainable from such sources.

Irradiation is the process of interception of radiant energy by an object. It may be considered as the converse of radiation. Thus, it is logical to speak of radiation by a source, in the sense of the process of the emission of radiant energy, and of the irradiation of an object. Irradiation may be specified in terms of the spectral region involved such as visible irradiation, ultraviolet irradiation, or X-irradiation; or in terms of the type of source used, such as incandescent lamp irradiation or solar irradiation. *Illumination* is the corresponding photometric term and is, therefore, the converse of lumination.

The quantitative expression of the degree or magnitude of irradiation is the *irradiance (irradiancy)*, which is the intensity term dealing with the interception of radiant energy. The irradiance of a surface is the incident radiant flux per unit area. It is very unfortunate that *intensity,* which is a general nonspecific term, has been used so extensively as a substitute for the specific term *irradiance.*

TABLE III

CONVERSION FACTORS FOR IRRADIANCE UNITS

UNITS	ERG/(SEC CM²)	ERG/(SEC MM²)	MICRO-WATT/CM²	GM CAL/(MIN CM²)
Erg/(sec cm²)	1.	0.01	0.1	1.43×10^{-6}
Erg/(sec mm²)	100.	1.	10.	1.43×10^{-4}
Microwatt/cm²	10.	0.1	1.	1.43×10^{-5}
Gm cal/(min cm²)	7×10^{5}	7×10^{3}	7×10^{4}	1.

While the defining equations and the basic units for radiant emittance and irradiance as given in table I are the same, it should be noted that the former term applies to sources while the latter applies to interceptors of radiant energy.

Many units of irradiance have been employed in the literature. Some of those units which have been used most frequently are compared in table III. The microwatt per square centimeter is a very convenient and logical unit for biological work. It is the unit which the National Bureau of Standards uses in expressing the irradiances obtainable with their standard lamps.

The photometric analog of irradiance is *illuminance (illumination).* It is expressed in such units as the lux, lumen per square meter or metercandle, the phot or lumen per square centimeter, and the footcandle or lumen per square foot. The relationships between these units are given in table IV. The footcandle is an unfortunate unit both as to name and definition because it implies the product of the foot and the candle and because it is not based upon the metric system of units. Since, however, it has attained such general usage in the United States and most of the commercially available illuminometers are calibrated in footcandles, it will undoubtedly continue to be used.

The terms *dark* and *darkness* designate a zero level of the psychophysical entity, luminous energy or light, whose absence is obviously without any

physical influence of any kind.   Since there is no convenient radiometric analog, the terms are logically carried over to imply a zero level of radiant energy in the near ultraviolet, visible, and near infrared regions of the spectrum, the limits depending upon the nature of the reactions under consideration.   The terms frequently have been used as if they referred to a positive entity capable of a specific physical or chemical influence.   Typical examples of such misuse are "the effect of light and darkness," "reactions due to darkness," and "dark reactions."   The term "dark reaction" refers to chemical or physical reactions which do not require the direct absorption of radiant energy, but which are associated with the photochemical reactions under consideration.   Such "dark reactions" do not require darkness in order to proceed nor are they usually affected by the incidence of radiant energy except indirectly as the precursors or the reaction products may be affected by the photochemical reactions.

TABLE IV

CONVERSION FACTORS FOR ILLUMINANCE UNITS

UNITS	LUX	PHOT	MILLIPHOT	FOOTCANDLE
Lux	1.	0.0001	0.1	0.0929
Phot	10000.	1.	1000.	929.
Milliphot	10.	0.001	1.	0.929
Footcandle	10.76	0.001076	1.076	1.

1 lux = 1 lumen incident per square meter;
        = 1 metercandle.
1 phot = 1 lumen incident per square centimeter.
1 footcandle = 1 lumen incident per square foot.

Experimentally it is never possible to exclude all forms of radiant energy.   Cosmic rays are always present and all bodies above a temperature of absolute zero are radiating energy which is largely confined to the infrared region of the spectrum.   While obviously it is seldom necessary to specify the spectral limits so completely, these considerations should be kept in mind in using terms implying zero levels of radiant energy.

Radiometry is the science of the measurement of radiant energy.   The instrument used for such measurements is known as a *radiometer* or a *photometer*.   The prefix photo- is used here in the general sense as previously mentioned.   *Spectroradiometry* or *spectrophotometry* is the science of the measurement of radiant energy as a function of the wavelength or frequency.   The instrument used for the measurement of the spectral energy distribution of the radiant flux is called a *spectroradiometer* or a *spectrophotometer* and consists of a radiometer or photometer in conjunction with a spectrometer.   The term *spectrograph* usually is applied to instruments which record the spectrum on a photographic emulsion, but the term has also been extended to those instruments in which the spectrum is recorded mechanically on a chart or electrically recorded with a cathode ray tube.   The more logical procedure is to term such instruments *recording spectroradi-*

ometers (**13**) or *recording spectrophotometers* (**2**) in order to distinguish them from photographic instruments.

Spectral terms

The *spectrum* is an optical arrangement of radiant energy with respect to wavelength or frequency. The *spectral* distribution of radiant energy is a function expressing analytically or graphically the relation between the radiant flux and the wavelength or frequency. The relationships between the various wavelength units are given in table V. They are the micron (μ), the millimicron (mμ), the Angstrom (A), and the X unit (XU). The millimicron is the most convenient unit for use in the visible spectrum where only

TABLE V

COMPARISON OF SPECTRAL UNITS

WAVELENGTH			FREQUENCY	WAVE NUMBER
MICRON μ	MILLIMICRON mμ	ANGSTROM A	FRESNEL f	WAVELENGTHS/CM cm^{-1}
0.2	200	2000	1500	50000
0.3	300	3000	1000	33300
0.4	400	4000	750	25000
0.5	500	5000	600	20000
0.6	600	6000	500	16600
0.7	700	7000	428	14300
0.8	800	8000	375	12500
0.9	900	9000	333	11100
1.0	1000	10000	300	10000

0.001 mm = 1 μ = 1000 mμ = 10,000 A = 10,000,000 XU.
The Fresnel, f, is equivalent to 10^{12} vibrations per second.
The wave number is equivalent to the reciprocal of the wavelength in centimeters.
The velocity of radiant energy *in vacuo* is 3.00×10^{10} cm./sec.

three significant figures are necessary. The millimicron sometimes has been designated inconsistently as $\mu\mu$. Since μ is intended to denote one-millionth, then the unit, $\mu\mu$, would be one-millionth of a micron instead of one-thousandth of a micron. It should be noted that the English A is now preferred in writing Angstrom; its abbreviation (A) replaces the Swedish Å. The X unit usually is used in the X-ray region of the spectrum.

Frequencies are expressed in units of the vibrations per second and in the fresnel (f). Another unit which is often used in place of frequency is the wave number which is the number of wavelengths per centimeter of path *in vacuo*.

Transmission and related terms

There is less agreement on terms relating to the transmission of radiant energy through matter than there is for the other phases of radiant energy nomenclature. The recommendations presented here are taken from correspondence with the chairman of the Optical Society Committee, the Illuminating Engineering Society report (**5**), and the United States Bureau of Standards Miscellaneous Publication 114 (**1**). Committees of other organi-

zations are now attempting to standardize this entire field of nomenclature and the present recommendations should be considered as tentative, especially in regard to the relationships governing the transmission of radiant energy through substances in solution which are given in table VI.

Transmission is the process by which radiant energy is transmitted through matter. *Diffuse transmission* is that form of transmission in which the transmitted radiant energy is emitted in all directions from the trans-

TABLE VI
RELATIONS GOVERNING THE PROPAGATION OF RADIANT ENERGY THROUGH SUBSTANCES IN SOLUTION

ABSORPTION CELL
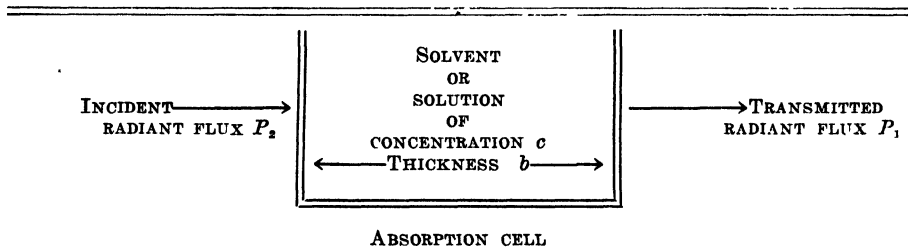
$$T_{solution} = \frac{P_2}{P_1} = \text{transmittance (transmission factor) of a cell containing the solution}$$

$$T_{solvent} = \frac{P_2}{P_1} = \text{transmittance (transmission factor) of the same (or a duplicate cell) containing the solvent}$$

$$T = \frac{T_{solution}}{T_{solvent}} = \text{transmittancy}$$

$$t = \sqrt[bc]{T} = \text{specific transmissivity (BEER's Law)}$$
$$b = \text{thickness of the solution}$$
$$c = \text{concentration of the solution}$$
$$k = -\log_{10} t = -1/bc \, \log_{10} T = \text{specific absorptivity}$$
$$i = -\log_e t = -1/bc \, \log_e T = \text{specific absorptive exponent}$$

mitting body. *Regular transmission* is that in which the direction of the transmitted pencil of radiant energy has a definite geometrical relation to the corresponding incident pencil.

Absorption is the process by which radiant energy is converted within a body into other forms of energy such as heat.

The *transmittance (transmission factor)* of a homogeneous body is the ratio of the radiant flux transmitted by the body to the incident flux. The transmittance takes into account not only the radiant energy which is absorbed directly by the body but also that which is reflected at the surfaces. Therefore it is the term which is used when no correction for surface reflection is made, *.*

The *transparence (transmittance)* is the ratio of the radiant flux incident to the second surface to that which enters the first surface. The use of this term takes into account only that radiant energy which is directly absorbed by the body itself and therefore is the transmittance corrected for surface

reflections.   When the regular transmission of a non-diffusing body such as a glass filter is measured in air by obtaining the ratio of the transmitted flux to the incident flux, the quantity that is obtained is the transmittance. The transparence of the same body can be obtained by making the measurement of incident and transmitted fluxes with the body immersed in a liquid having the same index of refraction as the body itself in order that surface reflections may be eliminated or at least reduced to negligible values.

Absorptance is the ratio of the radiant energy which is absorbed by a body to that which enters it.   It is obtained by subtracting the transparence from unity.

The *transmissivity* is concerned with solids and liquids of constant composition and is the transparence per unit thickness of the substance.

When dealing with substance in solution, it is desirable to correct for solvent absorption as well as reflection losses.   *Transmittancy* involves corrections for both of these losses and is applicable only to substances in solution.   It is usually obtained from the ratio of the transparence of an absorption cell containing the solution to that of an identical cell containing the solvent.   The transmissivity of such a solution is termed the *specific transmissivity* and is the transparence per unit thickness and concentration.   The adjective *specific* is added to terms involving substances in solution in order to distinguish them from analogous terms for homogeneous media in which concentration and solvent absorption are not factors.   The equation for specific transmissivity is an expression of BEER's Law which states that for solutions of constant thickness, the radiant flux transmitted by a substance in solution is an exponential function of concentration.   This law holds for dilute solutions but is frequently only an approximation for concentrated solutions.

The *specific absorptivity* is the common or decadic logarithm of the reciprocal of the specific transmissivity.   This quantity has been designated by various terms as specific absorptive index (1), extinction coefficient, etc. The natural or Naperian logarithm of the reciprocal of the transmissivity is the *specific absorptive exponent.*

Reflection is the process by which the direction of the propagation of radiant energy incident *at* a surface is changed.   In the case of *regular* or *specular* reflection, the angle of reflection is equal to the angle of incidence. In *diffuse reflection*, the radiant energy is reflected through all angles included within π radians or 180° for each small increment of surface.   The scattering of radiant energy from non-metallic films, such as surfaces of white paint pigments, usually involves both reflection and refraction, but the total process is spoken of as reflection and reflection terms are used.   *Reflectance (reflection factor)* is the ratio of reflected radiant energy to the incident radiant energy.

Refraction is the process by which the direction of propagation of radiant energy is changed in its passage *through* a surface boundary between two media.   The *refractive index* is the ratio of the sine of the angle of incidence to the sine of the angle of refraction.

Diffraction is the bending of waves around obstacles, the degree of bending increasing with the wavelength.

When radiant energy of one wavelength is absorbed by a substance and re-radiated at a longer wavelength, the phenomenon is termed *fluorescence.* If the re-emission of radiant energy persists for an appreciable length of time after the removal of the exciting energy, the term *phosphorescence* is usually applied to the phenomenon.

Miscellaneous terms

Polarized radiant energy, commonly called polarized light, is radiant energy in which the vibrations of the electromagnetic waves are confined to a single plane. In the case of non-polarized radiant energy, the vibrations are equally distributed in all planes about the axis of propagation. The energy emitted by most sources is non-polarized.

A *blackbody* is an ideal temperature radiator whose radiant emittance in all parts of the spectrum is the maximum obtainable from any temperature radiator at the same temperature. Such a radiator is called a blackbody because it will absorb all of the radiant energy which is incident to it. A *graybody* is a temperature radiator in which the radiant emittance is less than that of a blackbody at the same temperature. The ratio of radiant emittance of the graybody to that of a blackbody is the same at all wavelengths. This ratio is the *total emissivity* of the graybody and is always less than unity.

The *color temperature* of a source of radiant energy is that temperature at which a blackbody must be operated to give a color match with the source in question. For a source such as an incandescent tungsten filament, the spectral energy distribution may be calculated with considerable accuracy from published data on the spectral energy distribution of a blackbody having the same temperature as the color temperature of the lamp filament. Color temperatures are usually assignable only for sources which have a spectral energy distribution which is not greatly different from that of a blackbody within the visible spectrum.

The *quantum theory* postulates that the exchange of energy from one system to another is discontinuous in nature and that the energy is absorbed or emitted in small units known as *quanta.* The *quantum,* which also is called the *photon,* is therefore the smallest unit of energy which can be absorbed or emitted by an atom or a molecule. The energy of the quantum or photon is $h\nu$ where h is PLANCK's Constant, which is equal to 6.62×10^{-27} erg · sec, and ν is the frequency in vibration per second. Thus the energy of a quantum is proportional to the frequency and is inversely proportional to the wavelength.

EINSTEIN's principle of photochemical equivalence postulates that in a photochemical reaction the primary process involves the activation of one molecule of a substance by one quantum of radiant energy. The *einstein* is the energy required to activate one gram molecular weight of such a substance. The *quantum yield* or *quantum efficiency* of a photochemical reac-

tion is the number of molecules reacting per quantum of radiant energy absorbed or the ratio of the number of moles reacting to the number of einsteins absorbed.

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LITERATURE CITED

1. DAVIS, RAYMOND, and GIBSON, K. S. Filters for the reproduction of sunlight and daylight and the determination of color temperature. Bur. Standards Misc. Pub. 114. 1931.
2. HARRISON, G. R., and BENTLY, EDWARD P. An improved high speed recording spectrophotometer. Jour. Opt. Soc. Amer. **30**: 290–294. 1940.
3. Illuminating Engineering Society Report of Committee on Nomenclature and Standards Ill. Eng. Soc. Trans. **18**: 47–61. 1922.
4. Illuminating Engineering Society Report of Committee on Nomenclature and Standards. Ill. Eng. Soc. Trans. **28**: 263–279. 1933.
5. Illuminating Engineering Nomenclature and Photometric Standard. Ill. Eng. Soc. Trans. **36**: 815–852. 1941.
6. Illuminating Engineering Nomenclature and Photometric Standards. Ill. Eng. Soc. Report ASAZ 7.1. 1942.
7. IVES, H. E. The units and nomenclature of radiation and illumination. Astrophys. Jour. **45**: 39–49. 1917.
8. JONES, L. A. Colorimetry: Preliminary draft of a report on nomenclature and definitions. Jour. Opt. Soc. Amer. **27**: 207–213. 1937.
9. PRIEST, I. G. Colorimetry. Jour. Opt. Soc. Amer. **4**: 186. 1920. (Full report not published.)
10. Spectrophotometry: Report of the Optical Society of America Progress Committee for 1922–23. Jour. Opt. Soc. Amer. and Rev. Sci. Inst. **10**: 169–241. 1925.
11. TROLAND, L. T. Report of Committee on Colorimetry for 1920–21. Jour. Opt. Soc. Amer. **6**: 527–596. 1922.
12. TWENEY, C. F., and HUGHES, L. E. C. Chambers's technical dictionary. MacMillan. 1940.
13. ZWORYKIN, V. K. An automatic recording spectroradiometer for cathodoluminescent materials. Jour. Opt. Soc. Amer. **29**: 84–91. 1939.

SUGARS IN RELATION TO COLOR AND THIOCYANATE SPRAY IN APPLES[1]

R. H. Leonard and R. B. Dustman

Since the discovery of the effect of sodium thiocyanate spray in increasing the amount of red color in apples (3, 4) information has been desired concerning the chemical changes occurring in the sprayed fruit and the relationships of anthocyanins to other constituents. It has long been known that carbohydrates are associated with anthocyanins and related in some manner to their formation; consequently a study of the sugars present constituted the first step in seeking a possible explanation of this relationship.

Since the thiocyanate spray increases color development it is reasonable to suppose there may be other differences in chemical composition between sprayed and unsprayed fruits. The red pigment occurs in small and variable amount and cannot be measured quantitatively. For this reason color differences can be expressed in qualitative terms only, but the sugars can be measured and expressed in quantitative values.

The identity of the red pigment in three varieties of apples (2, 8) suggests that it may be the same material in all varieties, viz., idaein or 3-β-galactosidyl-cyanidin. Determination of the differences in the sugar content of various regions of the fruit (1) has shown that more sugar is present in the stem end than in the calyx end; and that there is more sugar in the colored side than in the uncolored side. Levulose has been found to be the preponderant sugar in the apple (5), but dextrose and sucrose also are present in considerable amounts. These three appear to be the only sugars present in the apple in significant quantities.

Materials and methods

The apples analyzed for this report were grown in the University Horticulture experimental orchard at Morgantown in the summer of 1941. All trees received the customary sprays for insect and disease control (9). Superimposed upon this treatment in certain varieties were three sprays of 0.1 per cent. sodium thiocyanate, the characteristic effects of which have already been described (4). All varieties were harvested in season and stored at 3° C. until analyzed. Summer varieties were analyzed in the early fall and analyses of the winter varieties were completed before the end of the year except those relating to storage studies.

SAMPLING

Samples showing both a red and a green side of approximately equal area were selected in the orchard from fruit with and without the thiocyanate spray. These samples were analyzed immediately after the apples

were harvested. In taking samples of tissue each individual apple was examined to determine the region of maximum color, halved from stem to calyx, the halves held tightly together and another cut made in the same manner at a rotation of a few degrees. This provided two small sectors one of which represented the region of greatest and the other the region of least color. The sectors were kept as uniform in size as possible and constituted about one-sixteenth to one-thirty-second of the fruit.

In comparisons of fruit involving the thiocyanate spray all fruit including both sprayed and unsprayed was always taken from the same exposure and the same large limb of the tree, thus giving the closest comparison possible. All samples analyzed were composites and usually included 25 to 30 apples of each variety.

Comparisons studied included composition of: (1) red versus green side of partly colored apples; (2) thiocyanate-sprayed versus unsprayed fruit; (3) peels from sprayed versus unsprayed fruit; (4) exterior versus interior tissue of sprayed and unsprayed fruit; (5) sugars by alcoholic extraction versus sugars in expressed juice; and (6) changes occurring in the sprayed and unsprayed fruit during storage.

ANALYTICAL PROCEDURE

The investigator has a wide choice of methods for sampling, extracting, and determining many of the constituents of plants. Even so he frequently combines features from several different procedures as being suitable for one reason or another. This has been done in the present work with sugars. All apples were cored with a no. 6 cork borer before samples of the tissue were taken. Suitable sectors were combined to form composite samples which were heated to 140° C. in aluminum dishes, ground in a Waring Blendor, extracted with alcohol and the extracts prepared and analyzed by the procedure already described (6). Dry matter was determined by drying for four and a half hours in a forced draft oven at 105° C. Titratable acidity was determined by electrometric titration of fresh tissue in aqueous solution with 0.1 N NaOH and pH also was determined electrometrically with the use of the glass electrode. The end point of the titration was taken as the point of inflection of the titration curve and occurred at average pH 8.5.

Results

RED VERSUS GREEN SIDE

In the red versus green side comparisons there were nineteen comparisons including eleven varieties with 10 to 160 apples from each variety, or a total of 436 individual apples. The varieties analyzed were Duchess, Wealthy, Red Duchess, Melba, King David, Winesap, York, Stayman, Jonathan, Rome, and Snow. Certain of the varieties provided samples of both thiocyanate-sprayed and unsprayed fruit. The average percentage composition of fresh tissue in the red and green sides of the eleven varieties

is shown in table I. In the table are given also the standard errors for the means, values for the ratios of red to green, and the odds for the differences observed as calculated from the paired samples (7). The mean ratios and odds shown in tables I, II, and III are calculated from the individually paired samples.

The results presented in table I show that dry matter, dextrose, levulose, and total sugar were higher on the red side of the apple. Sucrose, titratable acidity, and pH apparently were no higher on the red side although the value for pH approaches significance. Judging from the ratios, dextrose shows the greatest relative difference between the two sides.

TABLE I

DRY MATTER, SUGARS, AND ACID CONTENT OF THE RED AND GREEN SIDES OF APPLES

CONSTITUENT	AVERAGE OF ALL VARIETIES AND SAMPLES		PAIRED SAMPLES	
	RED SIDE	GREEN SIDE	MEAN RATIO OF RED/GREEN	ODDS
	%	%		
Dry matter	14.98 ± 0.50	14.07 ± 0.61	1.08 ± 0.02	86 : 1
Dextrose	1.69 ± 0.16	1.40 ± 0.12	1.21 ± 0.04	9999 : 1
Levulose	5.99 ± 0.19	5.66 ± 0.17	1.06 ± 0.02	499 : 1
Sucrose (as invert)	1.71 ± 0.19	1.65 ± 0.19	1.06 ± 0.03	12 : 1
Total sugar	9.39 ± 0.34	8.71 ± 0.32	1.08 ± 0.02	9999 : 1
Titratable acidity (as malic)	0.50 ± 0.03	0.50 ± 0.04	1.00 ± 0.02	1 : 1
pH	3.47 ± 0.03	3.45 ± 0.03	1.01 ± 0.00	18 : 1

SPRAYED VERSUS UNSPRAYED

The effect of the thiocyanate spray is indicated by eight comparisons from six varieties with 18 to 38 apples in each sample (total of 234 sprayed, and 207 unsprayed fruits). The varieties used were Melba, Wealthy, Rome, York, Stayman, and Jonathan. The average composition of the sprayed and unsprayed apples for the six varieties is given in table II, together with ratios and odds as given in table I.

Table II shows that dry matter, sucrose, titratable acidity, and possibly levulose were significantly lower in the sprayed than in the unsprayed fruit. The pH was highest in the sprayed fruit.

PEELS FROM SPRAYED VERSUS UNSPRAYED FRUIT

From a casual examination of sprayed fruits it appears that the metabolic change induced in the tissue by the thiocyanate spray occurs throughout the apple. In an attempt to find out if the spray might exert its influ- ence chiefly on the epidermis of the apple, some sprayed and unsprayed samples were stored for a short period after harvest. These apples were pared on an apple-peeler equipped with a circular knife which gave a uniform peel about 1 mm. thick. The peels were assembled in a sample and

TABLE II

DRY MATTER, SUGARS, AND ACID CONTENT OF SPRAYED AND UNSPRAYED APPLES

CONSTITUENT	AVERAGE OF ALL VARIETIES AND SAMPLES		PAIRED SAMPLES	
	SPRAYED	NOT SPRAYED	MEAN RATIO OF SPRAYED/NOT SPRAYED	ODDS
	%	%		
Dry matter	14.24 ± 0.57	14.82 ± 0.68	0.95 ± 0.02	150 : 1
Dextrose	1.43 ± 0.15	1.31 ± 0.18	1.11 ± 0.08	10 : 1
Levulose	5.85 ± 0.16	6.00 ± 0.20	0.98 ± 0.01	26 : 1
Sucrose (as invert sugar)	1.79 ± 0.25	2.06 ± 0.27	0.84 ± 0.06	99 : 1
Total sugar	9.05 ± 0.31	9.33 ± 0.42	0.98 ± 0.02	12 : 1
Titratable acidity (as malic)	0.40 ± 0.04	0.46 ± 0.04	0.87 ± 0.03	3689 : 1
pH	3.55 ± 0.05	3.44 ± 0.04	1.04 ± 0.01	999 : 1

treated in the same manner as the fleshy tissue, except that a longer period of grinding was necessary to disintegrate the peelings to a comparable state.

Twelve comparisons with eleven varieties were obtained with 8 to 53 apples in each sample. Peels were obtained from 380 sprayed and from 352 unsprayed fruits of Melba, Wealthy, Stayman, Maiden Blush, Red Duchess, Jonathan, McIntosh, Golden Delicious, Grimes, York, and Rome. The composition of the peels of sprayed and unsprayed fruits is given in table III.

Possibly two features are outstanding in the results shown in table III. One of these is that none of the sugars or dry matter is significantly lower in the peels from the sprayed fruit although the value for sucrose approaches significance. The other is that the titratable acidity is significantly lower in the peels of the fruit sprayed with thiocyanate, and the pH is correspondingly higher.

TABLE III

DRY MATTER, SUGARS, AND ACID CONTENT OF PEELS FROM SPRAYED AND UNSPRAYED FRUIT

CONSTITUENT	AVERAGE OF ALL VARIETIES AND SAMPLES		PAIRED SAMPLES	
	SPRAYED	NOT SPRAYED	MEAN RATIO OF SPRAYED/NOT SPRAYED	ODDS
	%	%		
Dry matter	20.15 ± 1.30	18.95 ± 1.22	1.07 ± 0.04	16 : 1
Dextrose	1.46 ± 0.14	1.42 ± 0.16	1.05 ± 0.04	4 : 1
Levulose	4.31 ± 0.31	4.37 ± 0.24	0.99 ± 0.03	2 : 1
Sucrose (as invert sugar)	1.76 ± 0.24	2.03 ± 0.29	0.87 ± 0.05	23 : 1
Total sugar	7.50 ± 0.48	7.80 ± 0.48	0.96 ± 0.03	8 : 1
Titratable acidity (as malic)	0.218 ± 0.021	0.291 ± 0.020	0.77 ± 0.06	332 : 1
pH	4.30 ± 0.07	4.12 ± 0.07	1.04 ± 0.01	20,000 : 1

EXTERIOR VERSUS INTERIOR TISSUE

The mechanism by which the spray affects the apple tissue is unknown. The thiocyanate may penetrate the epidermis of the apple or it may penetrate the epidermis of the leaves and be translocated to the fruit. With penetration through the apple skin differences might be expected between the outside and inside layers of tissue. If the translocation system was the chief means of transfer then differences between these two general regions would not necessarily be expected.

An attempt was made to determine whether the spray affected one region more than the other. By means of cork borers of suitable size a set of samples was obtained from Wealthy apples that included thirty apples each of sprayed and unsprayed fruit. A plug extending from the skin to the carpels was removed from exactly opposite sides. The outer third of this plug, measured along the length by inspection, was considered as representative of the shell of tissue at the exterior, and the inner third, not

TABLE IV

DRY MATTER, SUGARS, AND pH VALUE OF EXTERIOR AND INTERIOR TISSUES OF
SPRAYED AND UNSPRAYED WEALTHY APPLES

REGION	TREATMENT	pH	DRY MATTER	DEX-TROSE	LEVU-LOSE	SUCROSE (AS INVERT SUGAR)	TOTAL SUGAR
			%	%	%	%	%
Outer	Sprayed	3.39	11.15	1.11	4.81	0.39	6.31
"	Not sprayed	3.38	11.06	1.01	4.87	0.52	6.40
Inner	Sprayed	3.37	11.42	1.22	3.51	0.29	5.02
"	Not sprayed	3.38	11.34	1.11	3.45	0.27	4.83

including the carpel, was considered to be representative of the tissue of the inner region. The composition of the tissue from the two regions is shown in table IV.

A statistical analysis was not obtained but the results in table IV do not show a differential spray effect in the two general regions designated. Numerical differences between sprayed and unsprayed fruit are as great for the inner as for the outer region and the values vary only within the limit of error of the determinations.

A second comparison on possible regional differences was carried out in a somewhat different manner and was arranged to compare the epidermis with the layer of tissue directly beneath it. For this comparison thirty-five sprayed Rome apples and a like number of unsprayed fruits were pared with the circular-knife peeler. The outside peel was removed and the next adjacent layer was removed, thus giving two samples from each apple. The individual layers were combined into surface and sub-surface samples and analyzed with the results shown in table V.

In this trial table V shows that the spray apparently affected the peels in a slightly different manner than it affected the sub-surface. Spraying

TABLE V

DRY MATTER, SUGARS, AND ACID CONTENT OF THE SURFACE LAYER IN ROME APPLES
AS COMPARED WITH THE SUB-SURFACE LAYER

REGION	TREATMENT	pH	TITRATABLE ACIDITY (AS MALIC)	DRY MATTER	DEXTROSE	LEVULOSE	SUCROSE (AS INVERT SUGAR)	TOTAL SUGAR
			%	%	%	%	%	%
Surface	Sprayed	4.00	0.183	22.70	1.09	3.58	2.38	7.05
"	Not sprayed	4.00	0.189	21.09	1.23	4.50	2.17	7.90
Sub-surface	Sprayed	3.46	0.258	15.94	0.81	5.91	3.02	9.74
" "	Not sprayed	3.51	0.284	14.90	0.82	6.05	3.16	10.03

lowered titratable acidity more in the sub-surface than in the surface and increased the dry matter in the surface more than in the sub-surface layer. Levulose was lowered in the surface to a greater extent than in the sub-surface.

ALCOHOLIC EXTRACT VERSUS EXPRESSED JUICE

Prior to the present work numerous analyses had been made on fresh juice pressed from apples variously treated. It occurred to us that it might be of interest to compare sugar values obtained by alcoholic extraction with sugar values obtained from expressed juice. Two varieties, Melba and Wealthy, were sampled by the extraction procedure already described and the remnants were combined, run through a food chopper, and pressed immediately in a hand press without preheating. The extracts and the fresh juices were analyzed at the same time by the same analytical procedures with the results shown in table VI. Each value listed represents a composite sample of 24 individual apples for Melba and 30 apples for Wealthy.

The values given in table VI show that the results are not entirely consistent for the two varieties studied. Values for fresh juice are expressed

TABLE VI

SUGAR CONTENT OF APPLES AS DETERMINED BY ALCOHOLIC EXTRACTION AND FROM
EXPRESSED JUICE

VARIETY	TREATMENT	PORTION ANALYZED	DEXTROSE	LEVULOSE	SUCROSE (AS INVERT SUGAR)	TOTAL SUGAR
			%	%	%	%
Melba	Sprayed	Extract	0.90	4.75	2.41	8.06
Melba	Sprayed	Juice	0.59	4.59	2.53	7.71
Melba	Not sprayed	Extract	0.82	4.93	2.82	8.57
Melba	Not sprayed	Juice	0.35	4.62	3.11	8.08
Wealthy	Sprayed	Extract	1.17	4.16	0.34	5.67
Wealthy	Sprayed	Juice	1.06	5.12	0.08	6.26
Wealthy	Not sprayed	Extract	1.06	4.16	0.40	5.62
Wealthy	Not sprayed	Juice	0.84	5.21	0.08	6.13

as percentage of juice and might be expected to be slightly higher than those expressed as percentage of total tissue (extract). It might be expected, however, that both extract and juice although differing in total values would nevertheless also show the same relative proportions for the three sugars present. This is not the result obtained. In both varieties dextrose does not appear to have been as completely recovered by pressing as were the other sugars.

CHANGES IN COMPOSITION DURING STORAGE

A single series of storage trials involving but one variety was made on the effects of the thiocyanate spray on the composition of the fruit during storage. Large samples of sprayed and unsprayed Rome apples were taken from a single large limb on September 30, divided into six uniform lots of thirty-five apples each (three lots within each treatment) and placed in stor-

TABLE VII

DRY MATTER, SUGARS, AND ACID CONTENT OF ROME APPLES DURING STORAGE

TREATMENT	DATE OF ANALYSIS	PH	TITRA-TABLE ACIDITY (AS MALIC)	DRY MATTER	DEX-TROSE	LEVU-LOSE	SUCROSE (AS IN-VERT SUGAR)	TOTAL SUGAR
			%	%	%	%	%	%
Sprayed	October 2	3.54	0.323	12.75	0.86	5.77	2.09	8.72
Sprayed	Nov. 27	3.87	0.233	13.87	1.26	6.06	1.86	9.18
Sprayed	Feb. 2	3.96	0.221	14.03	1.40	5.92	1.62	8.94
Not sprayed	October 2	3.39	0.412	14.43	0.90	5.96	2.33	9.19
Not sprayed	Nov. 27	3.67	0.327	14.43	1.11	6.16	2.31	9.58
Not sprayed	Feb. 2	3.86	0.289	13.50	1.04	5.76	1.69	8.49

age at 3° C. Two days later the first lots were removed and sampled for analysis and the analyses were repeated on the remaining lots at bimonthly intervals. The results of these analyses are given in table VII.

Table VII shows that spraying caused a lowering of sugars at the beginning of the storage period. Here, also, spraying reduced the titratable acidity and increased the pH value, as reported in table II, and although the acid content fell continuously during the storage period the difference due to spraying was relatively the same throughout.

Discussion

In studying the effect of thiocyanate spray on the sugar content of apples it seemed desirable, since thiocyanate affects the amount of red color formed, to establish first the amount of difference, if any, to be expected for the several sugars between colored and uncolored sides of the same fruit. Analyses soon indicated that the thiocyanate spray did not widen this difference and consequently the data for sprayed and unsprayed fruit are combined in the values reported in table I. Where sufficient data were available (tables I,

II, and III) the significance of the differences was calculated. Cases with odds of 30 to 1 or higher are considered to be significant. Dry matter, dextrose, levulose, and total sugar were measurably higher on the colored side. Differences for sucrose are not significant but, interestingly, differences for dextrose are highly significant although dextrose is present in approximately the same amount as sucrose. Of the seven constituents determined, dextrose showed the greatest relative difference between the two sides.

The data in table II show that dextrose was the only sugar showing a higher value in the sprayed fruit, but the difference is not significant. In general, spraying with thiocyanate seems to have lowered most of the constituents determined. It should be stated that in all red varieties, spraying with thiocyanate increased the amount of red color exhibited by the sprayed fruit so that the sprayed samples always showed more red color than the corresponding unsprayed samples. Dextrose and pH tend to have the same relationship in the sprayed to unsprayed fruit as they did in the red to green side of the apples. The other constituents are contrary to what was anticipated (*i.e.*, are lower in the sprayed fruit) by reason of the higher color in the thiocyanate-sprayed fruit. Because of the increase in color it had been anticipated that a relationship between sprayed and unsprayed fruit would be found similar to that which exists between colored and uncolored sides of the same fruit. In this comparison the greatest sucrose content was present with the greatest acid content.

From table III it is clear that the thiocyanate spray had the same influence in lowering the titratable acidity and increasing the pH of the peels as was shown for the whole fruit in table II. Comparisons of peels with fruit in respect to other constituents show less clear cut agreement. It is of interest to note that dextrose and sucrose were present to about the same extent in both peels and whole fruit whereas levulose was about one and one-half per cent. lower in the peels.

The attempt to determine the effect of the thiocyanate spray on exterior and interior regions by means of plugs cut with cork borers failed to show appreciable differences (table IV). It was realized, however, that the regions represented were rather large and general, and a second effort was confined to a much smaller range of tissue. Table V gives the values obtained on two adjacent layers each about one mm. thick. The surface layer comprised the skin and a small amount of adhering fleshy tissue such as might be removed in ordinary mechanical peeling. The sub-surface layer was fleshy tissue only, adjoining the surface layer. As already mentioned this comparison indicates a differential effect on the layers examined. In an earlier publication (4) it was stated that "if leaves only are sprayed and the fruits protected, or if fruits only are sprayed and the leaves protected, a color response will be obtained in the fruit in either case, but one less pronounced than where both fruit and foliage are exposed to the action of the chemical agent." These data are in agreement with that observation to the extent that they do indicate some penetration through the skin and subsequent effect on composition as well as on color.

The differences in sugar composition as determined by alcoholic extraction and by analysis of the expressed juice are of interest. It is at once apparent that the absolute values would probably differ since they represent different products analyzed. As the three sugars are all readily soluble, however, the relative proportions of the sugars might be expected to be similar by both methods. Such an expectation is not borne out by the data. It should be stated that the Melba apples were harvested on August 2 at a full ripe stage and the analyses were begun on August 20, whereas the Wealthy apples were harvested on August 13 at a somewhat earlier stage and analysis started on August 26. Notations at the time of sampling describe the Melba as ''soft and mellow'' and the Wealthy as ''firm, hard and juicy.'' This difference in stage of maturity may help to explain some of the differences shown in table VI, as for example the much higher sucrose content of Melba. It will be noted, however, that levulose is higher in the extracts for Melba and higher in the juice for Wealthy, whereas sucrose is the exact reverse, being higher in the juice for Melba and higher in the extract for Wealthy. With both varieties dextrose seems to have been more completely recovered by alcohol than by pressing, resulting in a higher proportion of dextrose to total sugar when the sugars were removed by alcoholic extraction. The values should be regarded with caution as more data are needed for definite conclusions, but the point appears worthy of notice and perhaps of further investigation.

The outcome of the storage trials set forth in table VII shows that although the thiocyanate spray resulted in a lowering of sugar content at the time of harvest the sprayed fruit apparently did not use sugars as rapidly, as is evidenced by the fact that after four months in storage the sprayed fruit contained more sugars than the unsprayed. In a previous publication (4) it was stated that storage records indicated a superior keeping quality for the sprayed fruit. This observation has been confirmed further and may be partly explained by the present analyses on the basis of reduced sugar losses. Respiration studies of sprayed and unsprayed apples in storage would be helpful in clearing up this point.

Summary

1. A study of sugars and certain other constituents in relation to color and thiocyanate spray in apples has been made to include samples from fifteen varieties as follows: Duchess, Red Duchess, Wealthy, Melba, Winesap, King David, York, Stayman, Jonathan, Snow, Maiden Blush, McIntosh, Golden Delicious, Grimes, and Rome. All varieties except Winesap, King David, and Snow provided samples of both sprayed and unsprayed fruit, the three exceptions mentioned having been without thiocyanate treatment.

2. Red and green sides of selected apples showed differences in composition. Dry matter and sugars were higher on the red side, and dextrose showed the greatest relative difference between the two sides.

3. Spraying with sodium thiocyanate reduced the dry matter, sucrose, titratable acidity and possibly the levulose, and increased the pH value.

4. The thiocyanate spray reduced the titratable acidity and increased the pH value of the peels from sprayed fruit, but did not significantly affect the other constituents of the peels.

5. Evidence was obtained indicating that the thiocyanate spray is effective in part by penetration of the skin of the apple and resulting action upon the layers of tissue immediately beneath the skin. Evidence was also obtained indicating that the relative proportions of dextrose, levulose, and sucrose determined by alcoholic extraction of apple tissue may not be identical with those removed by pressing and determined in the juice.

6. Although spraying with thiocyanate caused a lowering of sugars in Rome at harvest time, after four months in storage the sprayed fruit actually had a higher sugar content than similar fruit not sprayed with thiocyanate.

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LITERATURE CITED

1. ARCHBOLD, H. K., and BARTER, A. M. Chemical studies in the physiology of apples. XV. The relation of carbon dioxide output to the loss of sugar and acid in Bramley's seedling apples during storage. Ann. Bot. **48**: 957–966. 1934.

2. DUNCAN, IRA J., and DUSTMAN, R. B. The anthocyanin pigment of the Winesap apple. Jour. Amer. Chem. Soc. **58**: 1511–1514. 1936.

3. DUSTMAN, R. B., and DUNCAN, I. J. Red color in apples. Science n.s. **90**: 233. 1939.

4. ——————— and ———————. Effect of certain thiocyanate sprays on foliage and fruit in apples. Plant Physiol. **15**: 343–348. 1940.

5. EOFF, J. R. Levulose the preponderant sugar of apple juices. Ind. & Eng. Chem. **9**: 587–588. 1917.

6. LEONARD, R. H. Sugars in relation to red color in apples. Thesis, The West Virginia University, 1942.

7. MILES, S. R. A new table of odds based on student's table of probability. Jour. Amer. Soc. Agron. **26**: 341–346. 1934.

8. SANDO, C. E. Coloring matters of Grimes Golden, Jonathan, and Stayman Winesap apples. Jour. Biol. Chem. **117**: 45–56. 1937.

9. SHERWOOD, E. C. Orchard pest control guide. West Virginia Agr. Ext. Circ. 320 (Rev.). 1940.

INFLUENCE OF UNKNOWN FACTORS ON THE VALIDITY OF MATHEMATICAL CORRELATIONS OF BIOLOGICAL DATA[1]

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(WITH THREE FIGURES)

Introduction

A constantly increasing use has been made of mathematical procedures in evaluating biological data during the past decade. Some of these procedures have been mathematically and biologically sound with a consequent advancement of scientific knowledge. On the other hand, there are many accumulations of biological data which do not lend themselves to usual statistical treatment. Attempts to establish statistical correlations in these instances lead to false conclusions which obscure all efforts to interpret the nature of the phenomena under consideration.

One of the most usual methods of investigating a biological problem is the observation of the effect of changes in some aspect of the environment on some measurable response of the organism. The word, "environment," in the present instance, is used with its broadest possible connotation, as signifying all factors and influences within the organism as well as external to it which can act to influence the response of the organism. The frequent use of graphs in the presentation of the relationships between cause and effect is sufficient evidence to indicate how universal is this approach to scientific problems.

The above sentences, at first hand, appear to state only a truism. Yet it is this approach that leads in many instances to erroneous conclusions. The purpose of the present paper is to call attention, as precisely as possible, to the manner in which these erroneous conclusions may enter into the interpretation of phenomena studied in this way.

It is rarely true that a pair of biological characteristics vary exclusively with each other. The more common situation is that each of the characteristics is influenced by a variety of others. For example, the weight of the grain yield of a wheat plant may increase with the size of the root system, but each of these in turn will be affected by the total leaf area, the nutrients in the soil, and many other factors. This interdependency of characteristics complicates the problem of the measurement of the degree of association between the two characteristics being studied; for example, the extent of the root system and the weight of grain produced.

One common statistical measure used in this situation is the PEARSON correlation coefficient. It is customary to subject these coefficients to a significance test in an attempt to determine whether or not they are of sufficient

magnitude to justify the hypothesis that they did not occur by chance. Such justification is said to exist, or not to exist, on the basis of a probability criterion. It is appropriate to point out that the purpose of such a significance test is the allowance for sampling variation, or error as it is often called. Indeed, if we assume the measuring process in the experiment to be perfect and there results a measure of correlation indicating lack of perfect association, then this lack of perfect association between the characteristics being studied would be attributed to variations in other factors in the biological system. The above statement, of course, assumes that the data satisfies all hypotheses required for the use of PEARSON's coefficient as a measure of correlation. Complete solution of the problem then requires the determination of the factors which affect the relationship in question. Failure to recognize the existence of such factors, and to measure or control their effect, frequently invalidates the use of common statistical procedures.

Effect of heterogeneity on the correlation coefficient

The simplest case in which other factors influence correlation coefficients as described above is that in which heterogeneity is present in the data, a situation difficult to avoid in biological research. The meaning of the term heterogeneity can best be explained by means of an example. In measuring the effect of a particular fertilizer on corn production two varieties of corn are used, one being naturally more productive than the other. The data resulting in this case will be heterogeneous due to the corn varieties. This particular situation can be avoided in this instance, but in a more subtle case, it might not be easily avoided. The importance of this heterogeneity is apparent from the following discussion in which it is shown how ordinary correlation coefficients may fail to measure the relationship between variables.

Let us suppose that variables A and B are each affected by some other factors or sums of factors. If all these other factors be held constant, that is, are controlled, three possibilities exist: (1) A and B are independent and the correlation is zero; (2) A and B are positively correlated; (3) A and B are negatively correlated.

In the first case a sample under control of associated factors would be expected to yield approximately zero correlation. In this case a graphical presentation of the data will show points grouped in a circular cluster if the variables are each measured in terms of one standard deviation as unit. Suppose that a second sample is taken with these other factors still controlled but at a level different from that of the first sample. Since A and B may be different in the two samples, four possibilities arise, the nature of which will be evident from figure 1. Each group of data exhibits zero correlation if it is collected under conditions which prevent a differential effect of other factors. For example, if A and B are characteristics of an agricultural crop, which are totally unrelated to each other, any number of samples collected under identical conditions yield approximately zero correlation
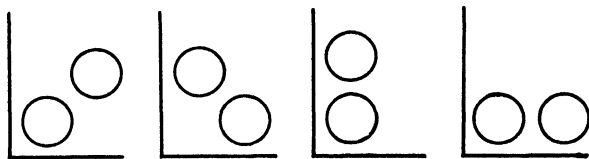
FIG. 1.

between A and B, chance variation from zero resulting from the sampling. If data obtained for two crops, however, or two harvests from the same planting, two separate fields, or under any other different circumstances are pooled, then spurious correlation is obtained. If unknown factors increase the means of both A and B, then the pooled data yield a positive correlation coefficient, indicating falsely that A varied because of some inherent relation with B if the correlation is interpreted in the usual way. If the mean of A is lessened, and that of B increased, then a negative correlation is obtained. If A or B simply remain unchanged, then zero correlation results.

In the second instance, suppose that any given group of data collected under identical conditions yields a positive correlation coefficient under all conditions indicating that A was inherently related to B. If two sets of
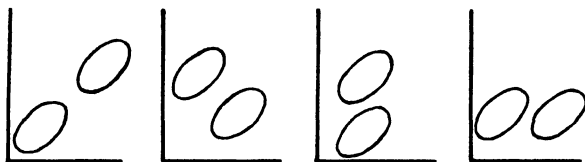
FIG. 2.

such data are pooled, false correlations are again possible, as is evident from figure 2. If unknown factors increase the means of both A and B, then the correlation of the pooled data will remain positive, but will exhibit an invalid increase in magnitude. It is equally evident that the pooled data may also yield a negative correlation.

In the third instance, groups of data, which are themselves always negatively correlated, may likewise yield spurious correlation coefficients when these groups are pooled. These possibilities are illustrated in figure 3.

The above illustrations show that it is difficult to measure by direct correlation analysis any inherent relation between A and B, even though such a relation may actually exist. Even though a sample yielded a positive correlation between A and B one still could not be certain that an increase in A
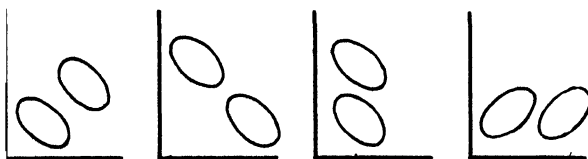
FIG. 3.

would be associated with an increase in B, other factors being held fixed. The correlation showing in the sample might be partly or completely the result of variation in other factors.

The only possible mathematical treatment would necessitate quantitative measurements of all factors which affect A and B in such a way as to permit partial and multiple correlation analysis. Unfortunately, in many biological problems, these various factors cannot even be well defined, and certainly are not measurable in any satisfactory quantitative manner. It should be pointed out that analysis of covariances measures significances of differences between independent estimates of the same covariances, but does not attempt to handle the problem discussed in this paper. It is, of course, possible to combine correlation coefficients of sets of data from the same population by a well known formula. Unfortunately variations in unknown factors implies the possibility of actually having more than one population.

Most variables of biological interest are affected by many factors, both known and unknown. Even when some of these factors are known, it may be impossible to control them or to measure them with any adequate degree of accuracy. For example, suppose that the effect of fertilizer treatments on the relationships between three variable aspects of an agricultural crop are being investigated. These variables could be any measurable response of the plant such as yield of dry matter, percentage of protein, and vitamin C content. The problem becomes one of determining what relationships exist between any two of these variables and how these are affected by the fertilizer treatment.

One may reasonably assume that other factors besides fertilizer will affect the variables A, B, and C. Small and localized differences in the soil, the slope of the field with its consequent effect on irrigation water, undetermined variations in climate, and many other factors certainly exert their respective influences on the physiology of the plant. Randomized arrangements of the plots, or selection of samples, do not eliminate this heterogeneity in the sum total of factors acting on the variables being studied. Neither do large samples, nor large numbers of samples avoid the disturbing effects of the heterogeneity of the environment. It follows that the smaller the area over which the plots are distributed, the more restricted is the time element during growth and sampling; and finally, strangely enough, the smaller the sample itself, the more likely will the sample represent a response conditioned specifically by the fertilizer treatments. It will be seen from the discussion below that this is not merely theoretically true, but is a demonstrable fact which may completely eliminate the possibility of treating any of the data by usual statistical methods.

The difficulty presented by the heterogeneity of the environment is inherent to the nature of biological problems and only under very special circumstances can it be avoided. There is an additional difficulty which further complicates efforts to analyze the data mathematically. Correlations between the variables may be numerically small, and a small number of data

may make it impossible to prove the significance of the observed value. The importance of establishing the magnitude of a correlation may be over-stressed by biologists. The nature of the biological problem frequently precludes the collection of a sufficiently large number of data to estimate accurately the magnitude of a correlation and for this reason, the observed value may be discarded as being insignificant; in reality, the correlation actually may be of the greatest importance. The fact that the magnitude of the individual correlation cannot be proved mathematically does not prove that it is therefore insignificant. Biologists frequently appear to be unaware that mathematical tests of significance give only probabilities of the existence of relationships but do not prove independence of the variables when the observed correlation is of insufficient size to justify the assumption of definite relationship on the basis of the given data.

Consistency of the algebraic sign as a test of correlation

In many types of biological research it is of value to determine the algebraic sign of relationships, even though the magnitude of the numerical value is not susceptible to direct proof. For example, if a calculation were based on 15 pairs of data, then a value of 0.64 would be sufficient to establish the existence of positive correlation with only one chance in 100 that a result at least this large would be obtained by pure chance. However, a set of 10 pairs of data between which there was a true correlation would require a value of 0.76 to prove its existence with the same degree of certainty. It thus appears that if a low correlation does exist, its presence may not be determined in a small group of data with a high degree of certainty because the significance test requires a large value which is itself unlikely in this case since the correlation was assumed to be low.

Even though the significance of any one correlation will remain uncertain, it is important to remember that knowledge of the existence of a correlation of one magnitude is just as valuable as that of any other magnitude in an effort to interpret the results. Even though the size of a correlation is valuable information, the problem of proving the existence of the correlation, in the probability sense, irrespective of its numerical magnitude, is likewise very frequently important. In other words, can it be determined that A and B are positively or negatively related, or that no relationship exists?

The following example illustrates the method by which such a decision may be reached. Let us assume that in a given group of data there is zero correlation, and that 10 pairs of data, drawn at random from this group, are studied by the usual methods of statistics. Suppose 10 successive similar samples of data have been drawn and their correlations computed and x are observed to be positive and $10 - x$ are negative. What is the probability of this result occurring by chance under the above hypothesis of zero correlation for $x = 0, 1, 2, \ldots, 10$? This problem may be translated into terms of drawing balls from a jar which contains an equal number of white and of

black balls.   Ten balls are withdrawn at random in' succession, and each replaced after it is withdrawn.   What is the probability that each of the possible number of white balls will be withdrawn in any group of ten withdrawals ?   Table I presents these probabilities.

The above probabilities that exactly the indicated number of white balls will be withdrawn are the terms of the expansion of the binomial expression, $(\frac{1}{2} + \frac{1}{2})^{10}$, and the probabilities that at least the indicated number will be withdrawn are the appropriate sums of these same terms.

On the basis of these probabilities, it is possible to determine whether any observed set of positive or negative correlation coefficients in any group of 10 different correlations is likely to occur by chance.   For example, if 9 signs are positive, then we would expect this result to occur only once in a hundred

TABLE I

PROBABILITIES THAT CERTAIN NUMBERS OF WHITE BALLS WILL BE WITHDRAWN FROM A HALF AND HALF MIXTURE OF WHITE AND BLACK BALLS

NUMBER OF WHITE BALLS WITHDRAWN IN 10 TRYS	PROBABILITY OF EXACTLY THIS NUMBER	PROBABILITY OF AT LEAST THIS NUMBER
10	1/1024	1/1024
9	10/1024	11/1024
8	45/1024	56/1024
7	120/1024	176/1024
6	210/1024	386/1024
5	252/1024	638/1024
4	210/1024	848/1024
3	120/1024	968/1024
2	45/1024	1013/1024
1	10/1024	1023/1024
0	1/1024	1

times in sampling from a population of an actual zero correlation.   If such a series should occur in the study of an accumulation of scientific data, we could state that there is positive correlation, and this statement would be true, in the long run, 99 times out of 100 such experiments.   The numerical magnitude of this correlation, of course, would remain unknown.   The larger the number of individual correlations, the more conclusive will be the results for any given number of signs.   It will be seen that we have applied a test for the consistency of the sign as an indication of the probability of the existence of a correlation bearing the same sign by means of refutation of a null hypothesis as is done in other cases.   The null hypothesis, that zero correlation actually exists, is refuted if the probability associated with the observed number of positive signs in the samples is small.   The hypothesis is not refuted if the above probability is large.   The definitions of large and small depend on the significance level which is chosen.

Investigation of heterogeneous biological data

Even though direct statistical correlations cannot yield the desired information concerning the inherent relationship between two variables, certain

facts about this relation can be determined by combining a study of the means of each variable under various conditions, the correlation coefficients of small groups of data, and the consistency of the algebraic sign of these coefficients.

In the study of the relationship between variables of the type discussed above, the investigator may be interested in two aspects of a problem. For example, the effect of fertilizer treatments, and also the effect of successive cuttings on the correlations between the responses A and B, A and C, or B and C. Thus data may be collected at a given time when presumably all factors except fertilizer treatment have been influencing equally all experimental plots, or data may be collected from successive harvests from the plots. In the first instance, all factors except fertilizer treatments are assumed to be constant; in the latter instance, the sum total of all other factors such as changes in climate, soil, and physiological state of the plant, in

TABLE II

CHANGES IN THE MEANS OF VARIABLES FOR HARVEST 1 TO HARVEST 2

	DIRECTION OF CHANGE OF THE MEAN	N	C	Y
For 18 correlation groups, 6 by re-combination of data	Increase	0	1	18
	Decrease	18	17	0
For 12 correlation groups, each on separate data	Increase	0	1	12
	Decrease	12	11	0

addition to the fertilizer, is free to operate on the characteristics A, B, and C of the crop.

An investigation similar to the above has been carried out by the writers. The details of the mathematical procedures involved are described below. The botanical features of the investigation will be presented in a future paper.

Measurement of three variables, N, C, and Y were made on each of two successive harvests from a series of plots receiving various fertilizer treatments and from a similar number of control plots. Three possible correlations were computed: r_{NC}, r_{NY}, and r_{CY}. During the course of the statistical analysis it was logical to make certain combinations of data as follows: for each harvest 12 correlations of each of the combinations of variables were obtained. By the combination of certain treatments, 6 additional correlations were calculated, giving 18 values of r_{NC}, r_{NY}, and r_{CY} for each cutting.

During the interval between the harvests, many unidentified factors were operating to change individually each of the 3 variables. These changes in the means of N, C, and Y are tabulated in table II.

It is clear that the means of N and C were less and that of Y was greater for the second harvest. Hence, unknown factors are decreasing N and C and are increasing Y over the period of time elapsing between the harvests.

These variations need not always persist in these directions for every lapse of time between the collection of field samples. Data to be published later show specifically that the changes in C do not always occur in the same direction with a lapse of time.

Now, if our interest is primarily concerned with relationships or correlations between N and C, N and Y, and C and Y it is clear that we must consider correlations and not means. This can be done best by considering all unknown factors affecting the means as a single new variable, X, with sign so chosen that its magnitude may be said to be increasing during the interval between the harvests. It is obvious that the choice of signs is no restriction to our statement that $r_{XN} < 0$, $r_{XC} < 0$, and $r_{XY} > 0$ from the evidence of the means alone, but we can say nothing about the correlations

TABLE III

THE NUMBER OF POSITIVE AND NEGATIVE CORRELATIONS OBTAINED FOR
VARIOUS SETS OF DATA AS INDICATED

NUMBER OF CORRELATIONS	HARVEST	r_{NC}		r_{NY}		r_{CY}	
		+	−	+	−	+	−
For 18 correlations, 6 by recombination of data	1st harvest	7	11	15	3	10	8
	2nd harvest	9	9	16	2	9	9
	1st and 2nd harvest pooled	17	1	3	15	2	16
For 12 correlations, each on separate data	1st harvest	5	7	9	3	7	5
	2nd harvest	5	7	10	2	6	6
	1st and 2nd harvest pooled	11	1	2	10	2	10

r_{NC}, r_{NY}, and r_{CY}. We wish to know the answers to the following three questions:

1. For a fixed X, what is the algebraic sign of r_{NC}, r_{NY}, and r_{CY}?

2. For another fixed X, will the correlation r_{NC}, r_{NY}, and r_{CY} have the same algebraic sign as for the first value of X?

3. Is there any method of obtaining information about the relationships, r_{NC}, r_{NY}, and r_{CY} under the hypothesis of allowing variations in X?

In order to answer the first two questions, we shall have to omit from consideration the correlations obtained from the pooled data from the two harvests, since the value of X was different for each harvest. An examination of table III shows that the correlations r_{NC} and r_{CY} are about equally divided as to sign, the divergences from equal numbers of positive and negative signs are so small as obviously to be likely due to chance variation. The correlations, r_{NY}, are predominantly positive in sign. Table IV gives the probability of the occurrence of at least as many positive correlations as were actually observed under the assumption that a zero correlation actually existed.

The probabilities, a to d, in table IV are those associated with the first two cuttings. They can be interpreted as follows. If there had been no

relationship between the variables, then by pure chance one would expect to obtain 15 or more positive correlations in a total of 18, about 4 times in 1000 experiments of this kind, each involving 18 random correlations. We are, therefore, justified in view of this comparatively low probability to assume that a relationship between the variable concerned probably does exist. The definition of a "sufficiently low" probability should depend to a large extent on the judgment of the experimenter. Certain conventional levels of significance are well known. If the correlations based on both harvests are considered, then a very significant result is obtained; namely, only 3 chances in 100,000 that 31 values of a given sign would be obtained in 36 correlations of inherently unrelated data. Such a combination seems justifiable.

There is yet the problem of answering the third question, concerning what can be said about r_{NC}, r_{NY}, and r_{CY} if X be allowed to vary. Since r_{XN} and r_{XC} agree with each other in sign, but disagree in sign with r_{XY} as seems

TABLE IV

PROBABILITY THAT THE OBSERVED NUMBER OF POSITIVE CORRELATIONS WERE OBTAINED BY CHANCE FROM A POPULATION OF A TRUE ZERO CORRELATION

HARVEST	NUMBER OF CORRELATIONS			PROBABILITY THAT AT LEAST THIS NUMBER OF POSITIVE CORRELATIONS WERE DUE TO CHANCE
	POSITIVE	NEGATIVE	TOTAL	
a 1st	15	3	18	0.004
b 2nd	16	2	18	0.0007
c 1st	9	3	12	0.07
d 2nd	10	2	12	0.02
a + b	31	5	36	0.00003*
c + d	19	5	24	0.003

* A conservative approximation by normal curve tables due to the size of the numbers involved.

likely from a study of the means, one would expect that if X is allowed to vary, r_{NC} would be positive while r_{NY} and r_{CY} would be negative. Table III shows that when data from both harvests are pooled, these results are actually obtained. For instance, the signs of r_{NC} are about equally positive and negative for any single harvest, that is to say, when X is constant; but when the data are pooled, the algebraic signs are 17 positive to only 1 negative. Table IV shows that such a result would be obtained by chance less than 7 times in 10,000 such experiments. It therefore appears reasonable to assume that the unknown factor X is effective in causing the observed positive sign of the correlations of the pooled data, N and C. The signs of r_{NY} are predominantly positive when X is constant, but 15 are negative and only 3 are positive when X is allowed to exert its influence. Table IV shows that this result would occur by chance only 4 times in 1,000 such experiments. Hence the unknown factor may be assumed to be the cause of a negative correlation of the pooled data, N and Y. The signs of r_{XY} are about equally positive and negative when X is constant, but 16 are negative and only 2 are positive when factor X is operating. Table IV shows that this distribution of alge-

braic signs would occur only 7 times in 10,000 such experiments which shows the effect of X in causing negative correlations of the pooled data, C and Y.

Summary

1. Groups of biological data obtained under different conditions cannot be pooled in an effort to increase the significance of statistical correlations by increasing the number of pairs of variates.

2. Direct correlation procedures are inadequate to prove or disprove inherent relationships between some biological characteristics, because factors, usually of unknown nature, may falsely exaggerate or diminish the numerical magnitude of the observed correlation coefficient. These factors may even change the algebraic sign of correlations obtained from the pooled data.

3. Under certain conditions, consistency of sign of a number of correlations may be used to establish relationships, even though the conditions of the experiment may preclude the usual statistical methods of establishing the numerical magnitude of any single correlation coefficient.

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BORDEAUX INJURY TO FOLIAGE AT LOW TEMPERATURES

C. E. YARWOOD

(WITH TWO FIGURES)

That bordeaux spray increases transpiration has been observed by many investigators (4). That bordeaux spray decreases leaf temperature has been demonstrated by TILFORD and MAY (5). These two facts singly or in combination might lead one to expect that bordeaux spray would increase the susceptibility of foliage to cold injury. The common observation that bordeaux injury to foliage, presumably copper injury, is sometimes greater during cool, wet weather, is probably unrelated to the subject under discussion.

Potatoes showing light late blight (*Phytophthora infestans*) infection were sprayed with bordeaux in two localities about a mile apart near San Juan, California, on September 28, 1941. In one of the localities the plants were severely and suddenly injured by some cause other than late blight, apparently frost, several days after spraying. Potatoes in the other locality were not severely injured. On October 16, the sprayed and unsprayed plants in the unfrosted locality appeared similar in vigor with about 75 per cent. of the leaves still living. At the same time in the presumably frosted locality the unsprayed plants had about 10 per cent. of their leaves living, while the bordeaux-sprayed plants had only about 2 per cent.

Dr. HEUBERGER of the Connecticut Agricultural Experiment Station informs the writer that he and Dr. HORSFALL observed that bordeaux-sprayed cantaloupe foliage was more severely injured by frost than unsprayed foliage in Connecticut in 1940. Also, Dr. RUEHLE of the Florida Agricultural Experiment Station writes that he has observed greater frost injury to bordeaux-sprayed than to adjacent unsprayed potatoes at Homestead, Florida. To the contrary, BUTLER (1) writes: "We are told that plants sprayed with bordeaux mixture resist white frost better than non-sprayed plants, which means nothing more, of course, than that sprayed leaves do not cool as rapidly as non-sprayed leaves." The matter of bordeaux injury as related to low temperature was therefore investigated under controlled conditions.

Materials and methods

For all tests 1 per cent. bordeaux was prepared by adding equal volumes of 10 per cent. $CuSO_4 \cdot 5H_2O$ and 10 per cent. CaO to the required amount of water. Plants were sprayed with an atomizer at 35 pounds air pressure and were used for subsequent tests within a few hours after the spray had dried. All tests were arranged in paired comparisons so that one set of leaves was sprayed with bordeaux and a similar set of leaves was left unsprayed. The pairs used for comparison were opposite primary leaves, as in some tests with beans, similar shoots from the same potato tuber, or simi-

lar plants.   All tests were replicated and repeated and were performed in the greenhouse, laboratory and cold rooms at Berkeley during the months of November, 1941, to December, 1942.

To measure transpiration, detached bean plants with their stems in cotton-stoppered flasks were placed in the dark test environment and weighed at intervals.   After completion of the test the leaf area was measured and the water loss was calculated on a unit area and time basis.   Leaf area was taken as the total plane surface area and is thus twice the measured planimeter area.   It is realized that the effect of bordeaux in increasing transpiration is sometimes greater for detached leaves than for potted plants (4), but this does not appear important for purposes of this study.

TABLE I

EFFECT OF BORDEAUX SPRAY ON LEAF TEMPERATURE AND LEAF INJURY AS RELATED TO LOW TEMPERATURE

TEST SURFACE	DEPRESSION OF TEMPERATURE; AMOUNT BY WHICH TEST SURFACE WAS LOWER THAN AIR TEMPERATURE			INJURY OF PLANTS HELD AT 0° C. FOR 4–12 HOURS AND THEN RETURNED TO GREENHOUSE
	DIFFUSE DAY LIGHT OF LABORATORY AT 21°, USUALLY ABOUT 50% R.H.	7° C. DARK ROOM 64% R.H.	0° C. DARK ROOM 70% R.H.	
	°C.	°C.	°C.	rating*
Wet thermometer bulb	– 6.1	– 2.5	– 1.4	
Young bean, unsprayed	– 2.3		– 0.3	
" " + bordeaux	– 3.5		– 0.6	
Medium bean, unsprayed	– 2 0	– 0.2	– 0.2	0.03†
" " + bordeaux	– 2.6	– 0.9	– 0.3	3.3†
Rusted bean, unsprayed	– 1.5	– 0.5		
" " + bordeaux	– 2.2	– 0.5		
Mildewed bean, unsprayed	– 1.5	– 0.1		
" " + bordeaux	– 2.0	– 0.5		
Potato, unsprayed	– 2.3			1.7‡
" + bordeaux	– 3.3			3.5‡

* Scale of 0 to 10 in which 0 indicates no apparent injury and 10 indicates leaves are dead.
† Each value is the arithmetic average of ratings of 7 plants.
‡ Each value is the arithmetic average of ratings of 14 shoots.

Temperature was measured to nearest 0.1° C. with glass thermometers with large cylindrical mercury reservoirs.   The thermometers were graduated to 0.2° C., and each was calibrated before use.   To measure leaf temperatures the test leaf attached to the potted plant was folded once around the mercury reservoir and held tightly in place with a clip.   In this way about 300 sq. mm. of the total of 366 sq. mm. outside area of the mercury reservoir was covered with and in tight contact with one thickness of leaf and the remainder, 66 sq. mm. was exposed to the environment.   One test was arranged so that the entire mercury reservoir was in contact with one layer of leaf and no difference in temperature from that obtained by the above

values were 0.11 of a gram loss and a ratio of 1.2, and in another 0.10 gram loss and a ratio of 1.5. The rate of water loss usually decreased rapidly with time at 0° C. Since these temperature comparisons were not performed under similar conditions of relative humidity and air circulation they should not be emphasized; but they do indicate that bordeaux spray increases transpiration over a wide temperature range, and even at 0° C. From the results secured it appears that the bordeaux ratio is less at high or low temperatures than at intermediate temperatures, and is always significantly greater than 1.

EFFECT OF BORDEAUX SPRAY ON LEAF TEMPERATURES

Bordeaux spray consistently reduced bean and potato leaf temperatures and the amount of reduction varied with the experimental conditions. A total of 948 records of leaf temperature and 316 of the corresponding air temperature were made. Some representative values are given in table I and some in the following discussion.

Temperature depression due to bordeaux was less at low than at moderate temperatures. This is indicated by the depression values of 2.6°, 0.9° and 0.3° C., for bordeaux-sprayed beans at 21°, 7°, and 0° C., respectively (table I). Because temperatures were read to 0.1° C. the relative error of temperature depression values was greater at low temperatures. The smaller temperature depression at low temperatures is to be expected from the lower actual transpiration and lower bordeaux ratios at low temperatures.

Young bean leaves with or without bordeaux spray showed more temperature depression than older leaves. This is indicated by the depression values of 3.5°, 2.6°, and 1.1° C. for young, medium, and old, bordeaux-sprayed bean leaves, respectively (table I).

When the lower surface of a bean leaf wrapped around a thermometer bulb was the transpiring surface exposed to atmospheric conditions, the temperature depression was lower than when the upper leaf surface was exposed. In one test the temperature of bordeaux-sprayed bean leaves was 2.7° C. below air temperature when the upper leaf surface was exposed to the atmosphere, and 3.6° C. below air temperature when the lower surface was exposed. This is presumably owing to the greater transpiration and resultant cooling of the lower leaf surface. Although the exposed surface of the leaf was the principal factor determining temperature depression, bordeaux treatment of the unexposed leaf surface against the mercury bulb of the thermometer also increased temperature depression somewhat, though no good reason for this is known. The data in table I were secured with the lower leaf surfaces exposed to the atmosphere.

The temperature depression of leaves in an air current of 450 feet per minute (about 5 miles per hour) was less than that of leaves in the relatively still air of the laboratory. In one representative test at 20° C. and 50 per cent. relative humidity, the temperature depressions for bordeaux-sprayed young, medium, and old bean leaves with the lower surface exposed to the environment were 2.8°, 1.7°, and 0.2° C., respectively, with an electric fan

turned on, and 3.5°, 2.3°, and 0.4° without the fan.   Other types of leaf sur-
faces gave similar differences.   In the same test as the above the tempera-
ture depression of the wet bulb was 6.9° with the fan turned on and 5.7°
without the fan.

In another test of this type a bean plant was attached to a photometer
and with one leaf wrapped around a thermometer.   The plant was held for
alternating 10-minute periods in still air, and in air at 600 feet per minute,
for a total of 90 minutes.   Each time the fan was turned on the leaf tem-
perature rose (as much as 1.3° C. in 2 minutes) and each time the fan was
turned off the leaf temperature fell, but no corresponding change could be
detected in the rate of transpiration.   These results are apparently the
opposite of those of CURTIS (2) who states ". . . fanning may cause a drop
in leaf temperature of from a few to 10° C. or more . . ." and ". . . tran-
spiration is often reduced when the shoot is vigorously fanned."   The expla-
nation is probably that CURTIS's observations were made in strong artificial
light while those of the writer were made in diffuse winter daylight in the
laboratory.   In the observations of CURTIS leaf temperatures were presuma-
bly above air temperature, while in this writer's tests of the type recorded
here, leaf temperatures were always below air temperature.

MILLER (4) has pointed out that most records of transpiration increase
due to bordeaux were with greenhouse-grown plants.   The writer believes
that the temperature depressions recorded above are due primarily to tran-
spiration and it might therefore be expected that temperature depression
would be greater for bordeaux-sprayed plants grown in the greenhouse than
for those grown outdoors.   All records of bean leaf temperature reported
here are for greenhouse-grown plants but a comparison was made of potatoes
grown outdoors with those grown in the greenhouse.   In one test in the
greenhouse on February 24, 1942, the temperature depression for control
and bordeaux-sprayed leaves was 2.9°, and 3.4°, respectively, for potatoes
grown in the greenhouse and 1.9° and 3.1° for potatoes grown outdoors.
This record indicates a temperature depression due to bordeaux for plants
grown outdoors, as well as for greenhouse plants.

Bordeaux also increased the temperature depression of bean leaves
infected with powdery mildew, *Erysiphe polygoni*, D.C., and of leaves
infected with rust, *Uromyces phaseoli* (Pers.) Wint. (table I).

EFFECT OF BORDEAUX AND LOW TEMPERATURE ON LEAF INJURY

Bordeaux-sprayed and control bean, potato, tomato, cucumber, canta-
loupe, and cabbage plants were exposed in the dark to 0° C. and about 70
per cent. relative humidity for 2 to 20 hours.   Except for cabbage, injury
resulted when bordeaux-sprayed plants were exposed at 0° C. for 12 hours
or more.   The controls were also injured in some tests but in every case
(total of 14 tests) the bordeaux-sprayed plants were more severely injured
than the unsprayed.   Cold injury was usually apparent as wilting while the
plants were in the cold rooms.   Wilted, unsprayed plants frequently recov-

ered when returned to the greenhouse but wilted sprayed leaves usually died. Control plants with similarly sprayed leaves left in the greenhouse continuously showed no apparent injury.

Average data for bean and potato, which were fairly sensitive to bordeaux injury at low temperatures, are given in table I and potato shoots from one test are illustrated in figure 1. That the injury was associated with water loss from the foliage and was not a direct injury due to bordeaux at the low temperatures tested is strongly indicated by the fact that bor-

Fig. 1.   Effect of bordeaux spray on cold injury to potatoes.   Shoots were on the same plant.   Shoot on right was sprayed 5 P.M., September 5, with 1 per cent. bordeaux. Shoot on left was unsprayed.   Both were exposed to 0° C. in the dark from 7: 30 P.M., September 5, to 9 A.M., September 6, and then returned to the greenhouse.   Detached and photographed 9 A.M., September 7.

deaux-sprayed and unsprayed beans at 0° C., but in moist chambers where transpiration was reduced or nil, showed no injury in 2 tests.

With greenhouse-grown tomatoes, exposure of bordeaux-sprayed plants to low temperature caused severe injury or killed the younger leaves, but caused no apparent injury to the older leaves in 2 tests.

Of the 6 plant species tested cantaloupe was apparently most sensitive to bordeaux injury at low temperatures.   Unsprayed cantaloupes exposed to 0° C. for 16 hours overnight were injured in all tests, and bordeaux-sprayed plants were usually killed by the same exposure.   To secure quantitative data the green weight of the leaves of sprayed and unsprayed plants

was measured 10 to 18 days after they had been subjected to a series of test temperatures for one overnight exposure. Average results are presented in figure 2. Exposures to 0°, 2°, and 7° C. greatly depressed the yield of unsprayed plants, and the yield reduction was even greater for the bordeaux-sprayed plants. At 13° to 34° C. temperature alone had no apparent effect on yield, and reduction in yield due to bordeaux was progressively less with increase in temperature. At 40° there was a fairly definite reduction in yield due to high temperature, with no apparent difference between bordeaux and control plants. At 0° to 2° C. the green weight of living leaves from bordeaux-sprayed plants was only 20 per cent. of the green weight of control plants, while at 28° to 40° C. the green weight of the bordeaux-sprayed plants was 98 per cent. of the green weight of the control plants. These results indicate that the bordeaux injury studied here is not merely an aggravation of injury caused by injurious temperature, but occurs only at low temperature.
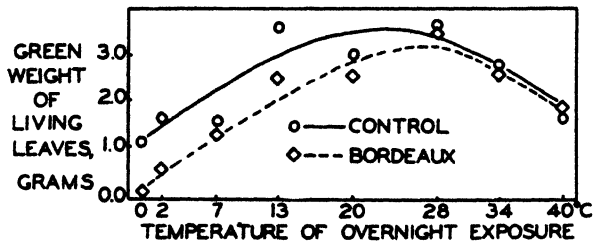
FIG. 2. Effect of bordeaux spray and exposure in the dark to a series of temperatures on the yield of cantaloupe plants. The potted plants were sprayed with bordeaux and allowed to dry, then placed at the test temperature for 16 hours from 4 P.M. to 8 A.M. and returned to the greenhouse. Ten to 16 days later all living leaves were weighed and the above is the average of 4 tests, with one control and one sprayed plant at each temperature in each test. The results clearly show that exposure of plants to low temperatures greatly decreased the weight of plants and that this reduction was greater on bordeaux-sprayed plants.

Cabbage was the only plant tested which did not show bordeaux injury at low temperatures. In one test, control plants and plants sprayed with 1 per cent. bordeaux plus 0.1 per cent. spreader were exposed for 16 hours in the dark to 0°, 2°, 7°, 13°, 20°, 28°, 34°, and 40° C. and no injury due to bordeaux or to low or high temperature was observed on any of the plants.

Discussion

The writer believes that the observed effect of bordeaux mixture in (a) increasing transpiration, (b) decreasing leaf temperature, and (c) increasing leaf injury at low temperature, are partially interdependent and that the two latter effects are partly the result of the preceding. To say that injury occurred at 0° C. as a result of increased transpiration and lowered leaf temperature due to bordeaux spray, is, of course, another way of saying that bordeaux-sprayed plants showed cold injury at higher air temperature than

unsprayed plants.   The low temperature injury considered here could logically occur only at relative humidities less than 100 per cent. and would therefore be less likely in a white frost than in a black frost.   It has not been determined in this study with potted plants whether the cold injury observed was the result of drying out of the leaf due to decreased root absorption at low temperatures (3) or due to other causes.   Since the injury occurred at temperatures far above the freezing point, however, it is obvious that the injury is not associated with the formation of ice in the leaf tissues, and cannot be frost injury.   Since wilting was an immediate symptom of bordeaux injury at low temperatures, it seems clear that the injury is directly associated with water relations.   The writer believes the injury was probably a result of the increased water loss due to bordeaux, associated with decreased absorption and translocation of water at low temperatures.

The importance of radiation in determining leaf temperatures in this study is not known.   It is commonly believed that radiation of leaf surfaces to the sky is largely responsible for their marked cooling below air temperature on clear nights.   No reported effect of bordeaux deposit on this radiation has come to the writer's attention, though CURTIS's observations [(2) fig. 4] indicate that lime deposit on leaves may have increased leaf radiation and decreased leaf temperature.   Unfortunately the only controls were the same leaves after the lime had been removed by wiping.   This effect of radiation may have been important in determining the difference in injury between bordeaux-sprayed and unsprayed plants in the field, but the writer does not believe that radiation was of importance in the laboratory studies reported here.

Bean, potato, cucumber, and cantaloupe plants, which showed bordeaux injury at low temperatures in these tests, would probably be horticulturally classified as sensitive to cold.   Cabbage, the most tolerant to cold under natural conditions of all the plants tested, showed no bordeaux injury associated with low temperature.   Though the numbers tested are small, it appears from the results that bordeaux injury associated with cold would be more pronounced on plants naturally sensitive to low temperatures.

Summary

In a field of potatoes apparently injured by frost, bordeaux-sprayed vines were more severely injured than the unsprayed.

In tests with greenhouse plants, bordeaux spray increased water loss from beans at temperatures from 0° to 37° C., decreased leaf temperatures of beans and potatoes by as much as 3.6° C., and caused injury to bean, potato, cucumber, and cantaloupe foliage at 0° C.   Unsprayed cantaloupe plants were injured by exposure to 0° to 2° C., but bordeaux-sprayed plants were more severely injured.   Control and bordeaux-sprayed cantaloupe plants exposed to 20° to 34° C. showed no marked bordeaux or temperature injury, and plants exposed at 40° C. showed temperature injury without bordeaux injury.   Cabbage plants showed no bordeaux or temperature injury at exposures of 0° to 40° C.

It is believed that the increased transpiration and decreased leaf temperature as a result of bordeaux spray are associated with the bordeaux injury to foliage at low temperatures.

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LITERATURE CITED

1. BUTLER, O. Bordeaux mixture II. Stimulatory action. New Hampshire Agr. Exp. Sta. Tech. Bull. 21. 1922.
2. CURTIS, OTIS F. Leaf temperatures and the cooling of leaves by radiation. Plant Physiol. **11**: 343–364. 1936.
3. KRAMER, PAUL J. Species differences with respect to water absorption at low soil temperatures. Amer. Jour. Bot. **29**: 828–832. 1942.
4. MILLER, EDWIN C. Plant physiology. McGraw-Hill Book Company, New York. 1938.
5. TILFORD, PAUL E., and MAY, CURTIS. The effect of bordeaux mixture on the internal temperature of potato leaflets. Phytopath. **19**: 943–949. 1929.

NUTRITIONAL RELATIONSHIPS OF BORON AND INDOLEACETIC ACID IN HEAD LETTUCE

ARTHUR D. MOINAT

(WITH TWO FIGURES)

Lettuce, which is being grown in ever increasing quantities, suffers from a number of nutritional disorders which have been the subject of considerable speculation and study. Much of the difficulty with lettuce is evidently the result of the type of forcing culture which is used to obtain the most firm and succulent heads. Among the micro-nutrient elements, boron has been given considerable attention in recent years since it is now known to be more widely deficient in the soils of the country than was believed earlier. For lettuce it has been shown that boron deficient plants show necrotic tissue near the leaf margins and over the growing point. Such plants have been photographed and described by McHARGUE and CALFEE (2) and others. Some have maintained that the symptoms are the same as those of the so-called tip-burn which has been the most serious disease of head lettuce in many localities. Perhaps that which the grower includes under the designation of tip-burn is in reality more than one physiological disorder. Mc-HARGUE and CALFEE found that 0.5–1.0 p.p.m. of boron in the culture medium was optimum for the production of lettuce leaf tissue dry matter, although they noted that quantities of boron which were toxic for young plants, might give favorable results with older plants.

The function of boron in the plant is unknown but several hypotheses as to its possible activities have been proposed. McMURTREY (3), in his review of nutritional deficiency symptoms, points out the importance of boron for proper functioning of the growing points. SCHROPP and ARENZ (5) found that a deficiency of boron was associated with greater uptake of nitrogen. Severe deficiency symptoms resemble those of ammonia poisoning. These authors also suggested an indirect function of boron, that of preventing swelling of the plasma colloids which might interfere with sap rise in the stem. MINARIK and SHIVE (4) found that, lacking an optimum boron supply, there was a subnormal quantity of calcium in the tissues of soybeans. SHIVE (6) has also reported a direct relationship between the soluble boron in plants and quantity of soluble calcium in both monocotyledons and dicotyledons. MINARIK and SHIVE found that within the range of boron concentrations used, the percentage of water in the tissues of soybean leaves decreased with increasing concentrations of boron in the nutrient solution.

A possible function of boron was suggested by EATON (1) as being a necessary element for the formation of an auxin, such as indoleacetic acid. He was of the opinion that those plants in his tests which were grown with a limited supply of boron showed an increased growth rate when indoleacetic acid was sprayed on the leaves. Similarly SKOOG (7) found an inter-rela-

tionship between zinc and indoleacetic acid suggesting the necessity of zinc for auxin synthesis or for the maintenance of auxin in an active state.

In the studies reported herein, a number of concentrations of boron in the nutrient solution for head lettuce formed one group of tests while across the boron series indoleacetic acid in several concentrations was applied to the leaves as a spray. With such a set-up it was hoped that we would be able to study the inter-effect of the two substances and also their relationship to the tip-burn disease.

Materials and methods

SERIES I

Lettuce of the variety New York was grown in sand cultures using HOAG-LAND's nutrient solution. The basic solution was made up with 0.005 M KNO_3, 0.005 M $Ca(NO_3)_2$, 0.002 M $MgSO_4$, and 0.001 M KH_2PO_4. Copper chloride was added to the basic solution at the rate of 0.02 p.p.m. Cu; $MnCl_2$ to get 0.4 p.p.m. Mn; $ZnCl_2$ to get 0.047 p.p.m. Zn. Ferric citrate was applied at the rate of 1 p.p.m. with each addition of nutrient solution to the sand. Nutrient solution was applied every second day or more often if necessary to prevent wilting. Each week the sand of each plant container was thoroughly flushed out with distilled water.

Boron was applied as boric acid in ten different concentrations of 0.0, 0.005, 0.025, 0.05, 0.10, 0.25, 0.50, 1.0, 2.5, and 5.0 p.p.m. of boron to as many groups of plants. Each group consisted of 21 plants, 3 plants to the container, and seven containers.

Indoleacetic acid was mixed with a lanolin-in-water emulsion, using sodium oleate as the emulsifying agent and making the following concentrations of indoleacetic acid: 0.0 (lanolin only), 5, 10, 20, 30, and 40 p.p.m. After the first three weeks of application, the concentrations of indoleacetic acid were increased to 0.0, 100, 150, 200, 250, and 300 p.p.m. The solutions were applied to the leaves and growing points of each plant each day with an atomizer. Of the seven pots of plants for each boron concentration, each pot of plants received a different concentration of indoleacetic acid spray including a distilled water spray for one pot of plants in each group.

On harvesting, the plant material was weighed and then reduced to dry weights in a forced draft oven at 80° C. Sand was separated from roots for dry weight determination by floating off the dried roots after a light grinding in a mortar. The plants were grown in earthenware jars in the greenhouse at the University of Chicago during the summer.

SERIES II

Plants for this group were grown in the greenhouse during the late autumn. Properly treated cans were used as the containers for the Ottawa sand. After the plants were one inch high, supplementary light from two 60-watt mazda lamps were given each night until harvest.

Six series of boron concentrations were used in the basic nutrient solution: 0.0, 0.001, 0.008, 0.10, 2.5, and 8.0 p.p.m. The six containers receiving

any one of the boron treatments were each given a different indoleacetic acid spray applied to the lettuce, the concentrations being 0.0, 100, 200, 250, and 300 p.p.m., the same solutions used in series I.   The boron concentrations used in the two tests varied somewhat, especially in the range of the optimum; but the exact determination of this datum was not one of the principal objects of the study.

Results

Series I plants grew rapidly but owing to the high temperatures or other unknown factors there was little tendency for heads to form.   The plants
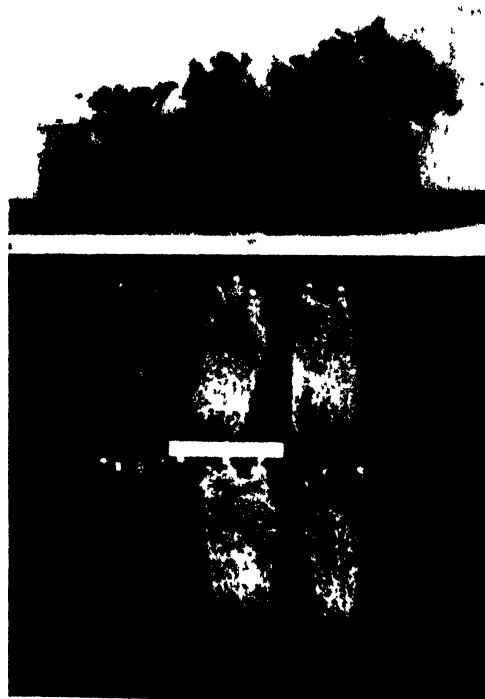
Fig. 1.   (Above) Shoot growth in the boron series.   Left to right: (1), minus boron, minus indoleacetic acid; (2), minus boron, 300 p.p.m. of indoleacetic acid; (3), 0.1 p.p.m. boron, 300 p.p.m. indoleacetic acid; (4), 5 p.p.m. boron, 500 p.p.m. indoleacetic acid; (5), 0.1 p.p.m. boron, minus indoleacetic acid.

Fig. 2.   (Below) Root growth in the boron series.   Upper row: shoot given daily spray of 300 p.p.m. indoleacetic acid; from left to right: minus boron, 0.1 p.p.m., and 0.5 p.p.m. boron in the nutrient solution.   Lower row without indoleacetic acid; left to right: minus boron, 1 p.p.m., and 0.5 p.p.m. boron in the nutrient solution.

developed a considerable amount of stem and the leaves tended to be less broad than is typical of lettuce grown in the field during cool weather (fig. 1).

As shown in table I, the addition of a very small quantity of boron (0.005 p.p.m.) to the nutrient solution produced a pronounced improvement in the

TABLE I

EFFECT OF BORON CONCENTRATION IN NUTRIENT SOLUTION ON THE GROWTH OF LETTUCE

SHOOT GROWTH

SERIES I

TEST	BORON CONCENTRATION†									
	0.0	0.005	0.025	0.05	0.10	0.25	0.50	1.0	2.5	5.0
Green weight (gm.)*	17.87	55.32	55.76	55.47	56.83	58.23	57.58	55.44	55.57	45.21
Dry weight (gm.)	2.05	4.53	4.42	4.23	4.26	4.43	4.28	4.03	4.07	3.79
Percentage H_2O	88.6	91.8	92.0	92.5	92.6	92.3	92.6	92.7	92.5	91.6

SERIES II

	BORON CONCENTRATION					
	0.0	0.001	0.008	0.10	2.5	8.0
Green weight (gm.)	4.77	14.64	72.9	89.05	108.36	83.37
Dry weight (gm.)	0.45	1.04	3.55	4.66	5.72	4.60
Percentage H_2O	90.8	92.8	95.2	94.6	94.6	94.5

ROOT GROWTH

SERIES I

	BORON CONCENTRATION									
	0.0	0.005	0.025	0.05	0.10	0.25	0.50	1.0	2.5	5.0
Dry weight (gm.)	0.75	1.85	1.71	1.86	1.47	1.93	1.77	1.61	1.52	1.34

SERIES II

	BORON CONCENTRATION					
	0.0	0.001	0.008	1.0	2.5	8.0
Dry weight (gm.)	0.04	0.12	0.21	0.35	0.58	0.28

* Yields are the average of each group yield; a boron group consisting of 7 indoleacetic acid treatments from 0.0 to 300 p.p.m.
† Concentration in parts per million.

fresh weights of the plants.   Five parts per million of boron were definitely toxic and caused a marked decline in the fresh and dry weights.   Boron in quantities of as much as 2.5 p.p.m. or 5 p.p.m. resulted in death of cells, and production of large brown bands along the margins of the older leaves.   The addition of 0.25 p.p.m. of boron to the culture solution appeared to be optimum for the production of green weight of leaves, although the differences in yields of dry or fresh weights for the range of boron applications between 0.005 p.p.m. and 2.5 p.p.m. were not significant.

Without boron added to the nutrient solution there were produced typical, thick, brittle leaves reduced in size and cupped in shape.   Brown spots and waxy exudations appeared on the younger leaves, and the growing point gradually became brown and non-functional.

In the series II, where plants were grown in the fall but with supplemental light, there was also little tendency to head; but the plants had shorter stems than did the summer grown plants.   The optimum supply of boron for fresh weight and dry weight of shoot material was at a concentration of 2.5 p.p.m., as is shown in table I.   Actually the optimum is probably somewhat below 2.5 p.p.m.   The differences in yields between the sets of plants supplied with different boron concentrations are all significant in series II.   Plants receiving but 0.001 p.p.m. of boron gradually developed deficiency symptoms, especially the cupping of the younger leaves, but these changes did not appear until about 8 days after the first deficiency symptoms appeared on the minus boron plants.

Root growth appeared to be optimum when the concentration of boron in the nutrient solution was 0.25 p.p.m. in series I and 2.5 p.p.m. in series II. Difficulties in obtaining net root weight make this determination very difficult in the case of lettuce.

Roots growing under conditions of boron deficiency were short and stubby with brown, rather than the usual white, growing points.   With limited growth, the area for absorption was necessarily decreased although the minus boron plants did not appear to wilt as easily as did the larger plants.   The smaller plants, of course, had a relatively greater water supply since all plants were given the same quantity of nutrient solution (fig. 2).

The percentage of water in the shoot tissues of plants of both series was slightly lower in plants grown in solutions with little or no boron supplied. Data showing this trend are given in table I.   Other differences in moisture content, except for the decrease in percentage of moisture in plants of series I, with an excess of boron are not of definite significance.   It would appear, however, that succulence or tenderness and turgidity of tissue are correlated with optimum nutrition and water supply, which result in rapid growth and large thin walled cells.

The lower concentrations of indoleacetic acid sprayed on the plants of series I daily for the first three weeks produced little change in the growth of the lettuce.   Certainly the treatment did not prevent the development of boron deficiency symptoms.   The stronger concentrations applied during

the last period of growth caused definite structural peculiarities such as epinasty of the leaves, thicker stems, and lighter colored stems and leaves. The higher concentrations did not prevent the development of boron deficiency symptoms in either series I or II. Although there was some indication of improved growth in series I (minus boron plants treated with indoleacetic acid) this was apparently the result of stronger plants at the beginning, or other factors, as the effect did not appear in the series II test. The data recorded in table II, giving the yields with various concentrations of

TABLE II

EFFECTS OF INDOLEACETIC ACID CONCENTRATION ON THE GROWTH OF LETTUCE

	SHOOT GROWTH						
	INDOLEACTIC ACID CONCENTRATION†						
TEST	SERIES I						
	0.0 D†	0.0 L‡	100	150	200	250	300
Green weight (gm.)§	57.44	58.01	53.15	54.77	55.05	57.02	51.31
Dry weight (gm.)	4.41	4.20	4.29	4.24	4.45	3.91	4.08
Percentage H₂O	92.5	92.5	91.8	92.3	91.9	92.9	92.1
	SERIES II						
Green weight (gm.)	....	65.82	70.35	60.11	58.6	64.02	54.23
Dry weight (gm.)	....	3.64	8.83	3.11	3.11	3.45	3.03
	ROOT GROWTH						
	SERIES I						
Dry weight (gm.)	1.53	1.66	1.70	1.56	1.54	1.70	2.02
	SERIES II						
Dry weight (gm.)	....	0.38	0.32	·0.24	0.17	0.18	0.23

§ Yields are the average of each group yield; each indoleacetic group consisted of 10 boron treatments from 0.0 to 5.0 p.p.m.
† D. Distilled water spray.
‡ L. Lanolin emulsion without indoleacetic acid.
* Concentration in parts per million.

indoleacetic acid, show no definite trends which can be ascribed to the auxin. Indoleacetic acid in series II apparently caused a somewhat more rapid elongation of the stems.

Boron deficiency symptoms are different from the so-called tipburn which causes much damage in the field. With the latter malady brown necrotic areas are most often found near the margins of leaves, averaging two or three inches in length. The brownish areas coalesce and tend to spread down the veins a short distance. Young plants, however, often show injury to the growing point as well as farther out on the leaves.

Typical tipburn, near the leaf margin, appeared on the plants of series I which were given boron solutions ranging from 0.005 to 2.5 p.p.m., but only on plants which did not receive the indoleacetic acid spray. One or two plants which were sprayed with indoleacetic acid in series II showed definite tipburn. In a third series of soil grown plants the tipburn appeared on

leaves sprayed daily with 250 p.p.m. of indoleacetic acid. Sand grown plants did not grow quite rapidly enough to produce the maximum quantity of tipburn.

Summary

1. As little as 0.005 p.p.m. of boron in the nutrient solution gave excellent growth of lettuce and prevented death of cells at the growing points as well as other deficiency symptoms. A concentration of 0.001 p.p.m. of boron delayed the appearance of deficiency symptoms.

2. Indoleacetic acid sprayed on the leaves produced plants with open heads, lighter green color, and shorter and thicker stems in summer grown plants; it did not, however, give evidence of replacing boron in promoting the normal growth and development which plants well supplied with boron have.

3. Neither boron nor indoleacetic acid prevented the appearance of the disorder known as tipburn.

4. The percentage of water in the shoots of the plants grown in a boron deficient medium was slightly lower than it was in the more normal plants grown with sufficient boron. A high boron concentration in the nutrient solution also resulted in a lower moisture content in the plant shoot.

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LITERATURE CITED

1. EATON, F. M. Interrelations in the effects of boron and indoleacetic acid on plant growth. Bot. Gaz. **101**: 700–705. 1940.

2. McHARGUE, J. S., and CALFEE, R. K. Further evidence that boron is essential for the growth of lettuce. Plant Physiol. **8**: 305–313. 1933.

3. McMURTREY, J. E. Distinctive plant symptoms caused by any one of the chemical elements essential for normal development. Bot. Rev. **4**: 183–203. 1938.

4. MINARIK, C. E., and SHIVE, J. W. The effect of boron in the substrate on calcium accumulation by soybean plants. Amer. Jour. Bot. **26**: 827–831. 1939.

5. SCHROPP, W., and ARENZ, B. Versuche mit Bor zu einigen Gramineen. Bodenk. u. Pflanzenernähr. **16**: 169–184. 1940.

6. SHIVE, J. W. Significant rôles of trace elements in the nutrition of plants. Plant Physiol. **16**: 435–445. 1941.

7. SKOOG, FOLKE. Relationships between zinc and auxin in the growth of higher plants. Amer. Jour. Bot. **27**: 939–951. 1940.

GERMINATION (EMERGENCE) OF VEGETABLE SEED AS AFFECTED BY DIFFERENT SOIL MOISTURE CONDITIONS[1]

L. D. Doneen and John H. MacGillivray

(WITH ONE FIGURE)

Moisture is one essential condition for seed germination, and poor germination is very costly to growers. There is, nevertheless, little experimental evidence concerning the effects of different percentages of available moisture (5) on germination; more study of the subject is essential. Since most vegetable seeds are planted at shallow depths, there may be rapid fluctuations of soil moisture around the seed, especially during the warmer months. This condition is common both to the irrigated soils of the west and to the soils of humid areas.

Though several reports have been published concerning the effect of soil moisture upon germination, in no case has there been accurate measurement of the available soil moisture. LIVINGSTON (3) indicates that seed germination varies with the species of plant concerned as well as with the percentage of soil moisture. According to COOPER (2), several species of forest-tree seeds respond differently to various amounts of supplementary water in addition to natural rainfall. BASS (1), studying the germination of New Zealand spinach, found it to be variable; but the germination was increased when the seeds were dried at intervals during the tests. PARKER and OLIVER (6) studied the combined effects of soil moisture and fertilizer placement on the germination of cabbage, snap beans, and pea seeds. In some treatments there were indications that low soil moisture and method of fertilizer application reduced germination. MACGILLIVRAY (4) has studied the effect of soil moisture, temperature, and varieties of wheat upon germination. Germination was affected by these three factors. As SHULL (7) has shown, the seeds of several plant species will absorb water from solutions much higher in atmospheric pressure than 16 atmospheres (8, 9) which is approximately the pressure of the permanent wilting percentage (5). Xanthium seeds proved able to extract water from a solution whose concentration was 1,000 atmospheres.

Methods and discussion

A Yolo fine sandy loam (field capacity 15.7 per cent., permanent wilting percentage 8.6 per cent.) and a Yolo clay (field capacity 29.9 per cent., permanent wilting percentage 14.9 per cent.), obtained on the University Farm, were used in this experiment because of their different ability to hold the available water. These soils were air dried, and the moisture contents determined. To obtain a series of soils with different moisture percentages, a quantity of soil was placed in a rotating cement mixer and was sprayed with

TABLE I

GERMINATION (EMERGENCE) OF VEGETABLE SEED IN YOLO FINE SANDY LOAM AT DIFFERENT SOIL MOISTURES, GROUPED WITH REFERENCE TO GERMINATION NEAR PERMANENT WILTING PERCENTAGE (FIELD CAPACITY 15.7 PER CENT.; PERMANENT WILTING PERCENTAGE 8.6 PER CENT.)

SEEDS TESTED	PERCENTAGE GERMINATION BY OFFICIAL METHODS	PERCENTAGE SOIL MOISTURE — GERMINATION									LEAST SIGNIFICANT DIFFERENCE
	%	7 %	8 %	9 %	10 %	11 %	12 %	14 %	16 %	18 %	%
Group A											
Cabbage, Copenhagen Market	93	0	80	94	95	92	93	93	91	86	7.7
Sunflower, dwarf		0	73	89	90	90	92	92	82	90	9.2
Turnip, Purple Top White Globe	92	0	72	89	88	91	91	87	90	87	7.7
Radish, Scarlet Globe	95	0	64	94	89	95	92	95	94	90	9.7
Summer squash, Zucchini	98	0	31	98	98	99	99	99	98	97	2.3
Sweet corn, Golden Bantam	95	2	35	90	95	93	93	89	93	95	6.9
Watermelon, Striped Klondike (Blue Ribbon)	86	1	39	82	83	83	84	87	85	85	8.8
Tomato, Essar	97	0	31	79	88	95	93	95	91	93	8.4
Winter squash, Hubbard	99	1	22	86	94	93	96	96	95	96	8.9
Cantaloupe, Mildew Resistant #45	99	0	7	92	99	97	99	97	97	96	3.7
Pepper, Pickling Wax	89	0	19	75	75	73	76	79	80	74	9.6
Cucumber, Short Colorado	—	—	0	84	97	99	98	98	99	98	4.7
Onion, Yellow Sweet Spanish	96	0	0	75	90	91	90	91	91	91	7.6
Spinach, Viroflay	87	0	0	74	90	93	94	94	95	85	6.1
Group B											
Carrot, Imperator	91	0	3	57	75	87	76	78	77	78	10.4
Snap beans, Stringless Greenpod	82	0	0	57	80	86	92	89	88	89	7.5
Spinach, New Zealand (seed balls)			4	42	64	76	81	83	80	61	8.7
Spinach, New Zealand (seedlings)		0	5	63	87	117	119	141	132	96	16.6
Group C											
Lettuce, Hanson	93	0	0	29	65	81	91	91	90	88	8.2
Lima beans, Baby Potato	88	0	0	23	79	89	86	86	89	91	5.5
Peas, Laxton's Progress	91	0	3	19	73	86	87	89	86	90	16.6
Beets, Detroit Dark Red (seed balls)	91	0	3	4	52	82	90	91	93	92	6.0
Beets, Detroit Dark Red (seedlings)		0	3	5	77	129	156	167	179	172	32.7
Group D											
Celery	80	0	0	0	0	29	43	62	73	82	

sufficient water to bring it up to the desired moisture level. In the case of the Yolo fine sandy loam it was possible to bring and hold the soil moisture above the moisture equivalent (18 per cent., table I). Some puddling of the soil was noted at this moisture content. By this method it was possible to obtain, within the limits of ± 0.2 per cent., the soil moisture desired. At and above 10 per cent. soil moisture for Yolo fine sandy loam and 20 per cent. soil moisture for Yolo clay, some of the crop seeds were treated with suitable seed disinfectants to obtain maximum germination. Seed treatments were not used at low soil moistures because they reduced the germination. With most of the crops 350 grams of the soil was placed in a no. 2 friction-top can, the soil was tamped, and 10 seeds were spaced evenly on the soil surface. Fifty grams of soil were used to cover the seed, and the surface again tamped. The larger seeds were placed on 300 gm. of soil and covered with 100 gm., so that in all cases each can held 400 gm. Twenty cans, or 200 seeds, were used for the averages of the Yolo fine sandy loam, and ten cans with 100 seeds for the Yolo clay. The seeds in these cans were germinated in a constant-temperature room at 77° F. except celery, lettuce, onions, and peas which were placed at 64° F. "Germination" as used in this paper indicates the percentage of seeds that sprouted and from which the plant emerged above the soil surface. Germination was recorded daily from the second to ninth day after seeding, and every other day for the remainder of the three-week germination period.

To obtain satisfactory germination, the lettuce seeds were planted $\frac{1}{8}$ of an inch deep in the soil, but otherwise were handled in the usual way. Celery did not give satisfactory germination under the same conditions, but germinated well when planted in the usual soil mixture in common Petri dishes. One hundred seeds were planted in each dish. For celery, better germination than that shown in table I could be obtained by adding a drop of water to each seed after planting or by covering the soil surface with a wet filter paper or a wet cloth. Under these conditions 100 per cent. germination was obtained in a single Petri-dish trial.

At the end of the experiment all cans were screened so the seeds could be examined and the sprouting noted. Sometimes the percentage of moisture was determined after screening. For beets and New Zealand spinach, both the germination of the seed balls and the number of seedlings per 100 seed balls were recorded. Sunflower seeds were included with the vegetables because they are frequently used in determining the permanent wilting percentage.

Based on these data, the vegetable crops seem to fall into four groups. The classification was made by using the percentage germination just above the permanent wilting percentage; in other words, 9 per cent. for the loam and 16 per cent. for the clay soils. Thus in group A the total germination covered a range of 98 to 74; in group B, 57 to 42; in group C, 29 to 4; and in group D, 0. Winter squash in table II was the only vegetable that failed to meet these criteria.

Vegetable seeds give a satisfactory field germination percentage over the entire range of available soil moisture except for lima beans, beets, celery, lettuce, peas, and New Zealand spinach, which germinated less than 50 per cent. near the permanent wilting percentage.   In addition, snap beans, carrots, and winter squash (clay soil only) seeds germinated less than 70 per cent.   With some of the crops there was considerable germination in both soils below the permanent wilting percentage.   The germination at low soil moisture appeared not to be correlated with the size of seeds.

. The germination of celery seeds was very striking because none was obtained below 11 per cent. soil moisture, which was well above the permanent wilting percentage.   This likely accounts for a common greenhouse practice
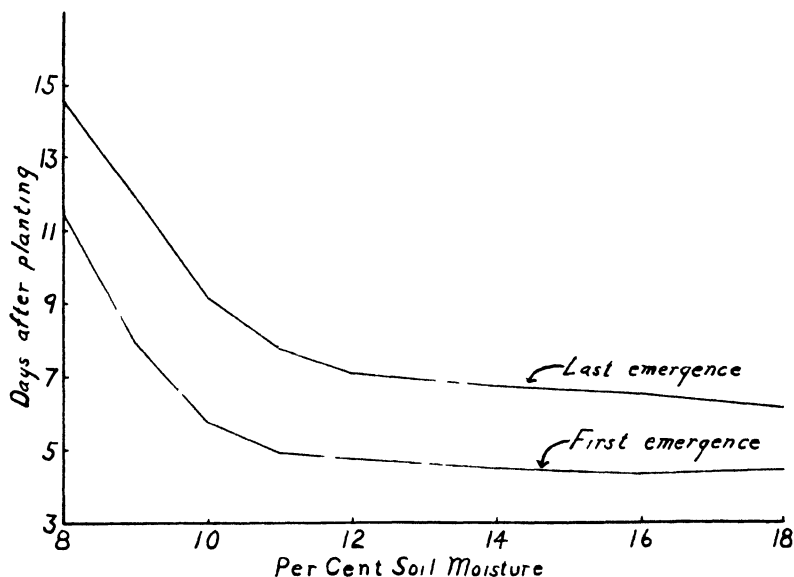
FIG. 1.   Interval in days between planting and emergence of first and last seeding. Average of fifteen crops planted in Yolo fine sandy loam.

of starting celery plants by scattering the seeds on the surface of the soil in a greenhouse flat, and then screening soil on the seeds until about half of them are covered.   To insure high moisture, the flat is watered frequently through a piece of burlap sack and may also be covered with a piece of window glass.   By keeping the burlap wet, the seeds are germinated in wet soil at a high humidity.

Seeds germinated more quickly at high soil moistures than at low and, except for the 8 per cent. soil moisture, the interval between first and last germination increased as the water content decreased.   All crops that germinated at 8 per cent. soil moisture and above were used for figure 1.   The soil moisture was determined in several cans of 9 per cent. and 10 per cent. series after the germination period of three weeks, to determine whether all the available moisture had been absorbed by the seeds.   For a representative sample of the crops, the soil moisture was reduced below the permanent

wilting percentage in 38 per cent. of the cases in the 9 per cent. soil moisture, and in only 13 per cent. of the cases in the 10 per cent. soil moisture (table I). In all trials in this work, the soil moisture given is that of the soil at the time of seed planting, and the reduction in soil moisture was critical only at low soil moistures.

Though the seeds of certain crops had sprouted at the low soil moistures, the plant did not appear above ground and so was not counted in the germination. Crops that germinated less than 80 per cent. at 9 per cent. soil moisture and that had a fair percentage of sprouted seed not appearing above the surface were lima beans (61 per cent.), beets (31 per cent.), and peas (68 per cent.). Lettuce had only 3 per cent. sprouted seed at 9 per cent. soil moisture. Many of these crops germinated less at the highest soil moistures than at some lower moisture, but the differences were significant only for spinach and New Zealand spinach.

TABLE II

GERMINATION (EMERGENCE) AT 77° F. OF VEGETABLE SEEDS IN YOLO CLAY SOIL AT DIFFERENT SOIL MOISTURES, GROUPED WITH REFERENCE TO GERMINATION NEAR PERMANENT WILTING PERCENTAGE. (FIELD CAPACITY = 29.9 PER CENT.; PERMANENT WILTING PERCENTAGE = 14.9 PER CENT.)

SEEDS TESTED	OFFICIAL GERMINA- TION	PERCENTAGE SOIL MOISTURE								LEAST SIGNIFICANT DIFFERENCE
		14	16	18	20	22	24	26	28	
		GERMINATION								
	%	%	%	%	%	%	%	%	%	
Group A										
Corn, sweet	95	38	80	95	91	87	95	91	90	3.4
Cucumber	90	30	91	90	85	88	84	82	84	19.9
Radish	95	2	85	96	93	93	95	95	92	8.2
Squash, winter	99	0	55	71	63	79	85	81	82	26.2
Tomato	97	11	93	89	91	95	89	91	87	8.4

Germination in closed tin containers was probably more favorable for some seeds than for others. All seeds germinated within 5 per cent. of the official germination except carrots, onions, and peppers for the sandy loam; and winter squash for the clay soil. In preliminary trials there was some damping-off or rotting of seedlings in the cans. This was reduced and greater germination obtained by proper seed treatments; but sterilized soil was not used because steam sterilization may affect the moisture-holding capacity of the soil.

For statistical analysis the percentage of germination was calculated on the basis of the 10 seeds in each can. At first it was planned to use 10 cans of each vegetable and each treatment. Since, however, the results were variable, the number of cans was increased to 20. Even then the errors were much greater for some vegetables than for others. The large error for cucumbers and winter squash on the clay soil (table II) is probably due in part to the use of only 100 seeds in 10 cans.

With the normal procedure it was impossible to obtain satisfactory germination of lettuce, celery, endive, and parsnips. Some of these crops have been mentioned already. Endive gave as high as 62 per cent. germination (official germination, 95 per cent.) at the higher figures of 14 to 18 per cent. soil moisture. Parsnip seeds gave poor germination at all soil moistures in cans; the official germination was not known. Endive and parsnips were not included in the tables; they may be typical of seeds found in group C or D.

Summary

Most vegetable seeds gave good germination over the entire range of available water and seem to fall into four groups based on their ability to germinate near the permanent wilting percentage. Seeds of all crops germinated in a shorter time at high soil moistures than at low.

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LITERATURE CITED

1. BASS, C. M. Methods of germinating New Zealand spinach. Proc. Assoc. Off. Seed Anal. No. Amer. Twenty-first Ann. Meet., pp. 36–37. 1929.
2. COOPER, J. R. Forty-third Ann. Report. Arkansas Agr. Exp. Sta. Bull. 268. 1931.
3. LIVINGSTON, B. E. The relation of desert plants to soil moisture and to evaporation. Carnegie Inst. Washington Pub. no. 50: 40–41. 1906.
4. MACGILLIVRAY, JOHN H. The effect of soil moistures and of temperatures on the germination and growth of varieties of wheat. Thesis, Graduate School, University of Illinois, 1922.
5. ————————, and L. D. DONEEN. Soil moisture conditions as related to the irrigation of truck crops on mineral soils. Proc. Amer. Soc. Hort. Sci. **40**: 483–492. 1942.
6. PARKER, M. M., and R. C. OLIVER. The effect of fertilizer placement, as influenced by soil moisture, on seed germination. Proc. Amer. Soc. Hort. Sci. **36**: 533–536. 1939.
7. SHULL, C. A. Measurement of surface forces in soils. Bot. Gaz. **62**: 1–31. 1916.
8. VEIHMEYER, F. J., and HENDRICKSON, A. H. Soil moisture at permanent wilting of plants. Plant Physiol. **3**: 355–357. 1928.
9. ————————, and ————————. Some plant and soil moisture relations. Amer. Soil Sur. Assoc. Bull. **15**: 76–80. 1934.

INFLUENCE OF THE HYDROGEN–ION CONCENTRATION OF THE SUBSTRATE ON THE DEVELOPMENT OF LEAFY MOSS PLANTS[1]

SAMUEL L. MEYER AND CLIFFORD H. FORD

Introduction

The literature on the physiology of mosses contains interesting results of investigations concerning the influence of the hydrogen-ion concentration of the substrate on the germination of moss spores, the growth of protonemata, and the development of leafy plants. In many cases, the conclusions reached are in marked disagreement.

TREBOUX (7) observed that the spores of such acid substrate mosses as Sphagnum and Dicranella germinate best in an acid medium. KESSLER (3) reported that the spores of mosses which grow under basic conditions germinate on an alkaline substrate while spores of mosses which grow in an acid environment germinate on a medium of similar reaction. PRINGSHEIM (5) concluded that though the protonemata of *Leptobryum piriforme* grow best in a slightly acid solution buds develop best in an alkaline medium. SCHWEIZER (6) obtained some interesting results with *Funaria hygrometrica,* a species which naturally occurs on alkaline or only slightly acid substrates. He found that spores can withstand a pH of 1.55 without impairment of germination; that protonemata develop equally well whether the acidity of the medium is high or low; and that young moss plants develop equally well in cultures of high or low acidity. IKENBERRY (2) studied the hydrogen-ion concentration of the habitats of various moss species as well as the pH values of solutions most favorable to spore germination and development of protonemata. He concluded that: "In general, there is no consistent correlation between the pH of the substratum where a given species is found in greatest abundance in nature and the reaction of the culture solution in which its spores germinate and protonema grow most readily." APINIS (1) observed that in order for protonemata to give rise to leafy plants: "It is necessary that the pH of the solution correspond to the pH interval observed in natural conditions." He concluded that in *Sphagnum plumosum, Funaria hygrometrica,* and *Polytrichum juniperinum:* "It was experimentally found that the pH interval of the leaf-bearing moss development or its optimum corresponds to the acidity of the substratum of the respective mosses in natural conditions." This conclusion is of particular interest when compared with the results of SCHWEIZER (6), mentioned above. APINIS cited the work of TREBOUX (7), KESSLER (3), PRINGSHEIM (5), and IKENBERRY (2) but apparently overlooked the contribution of SCHWEIZER (6). APINIS, however, very aptly summed up the

entire situation when he said: "It is apparent from the above that there is a certain uncertainty about this matter."

The present investigation was begun in order to determine the relation, if any, between the hydrogen-ion concentration of the substrate and the development of leafy moss plants from primary protonemata.

Materials and methods

For this investigation, eight buffered culture solutions were prepared in the manner described by IKENBERRY (2). A nutrient solution was prepared with the following composition: $Ca(NO_3)_2$ $4H_2O$, 0.491 gm.; $MgSO_4 \cdot 7H_2O$, 0.056 gm.; $Fe_2(SO_4)_3$, trace; and distilled water to 1000 ml. To a given amount of the nutrient solution, an equal quantity of phosphate buffers was added. The latter were combined in various proportions to give solutions of different pH values. The phosphate buffer mixtures were prepared from the following stock solutions: H_3PO_4, 2.178 gm. per liter; KH_2PO_4, 3.026 gm. per liter; K_2HPO_4, 3.872 gm. per liter; and K_3PO_4, 4.718 gm. per liter. A solid substrate nutrient medium was prepared by using the above mentioned solutions and 2 per cent. washed agar.

Eight series of cultures were set up in 125-ml. Erlenmeyer flasks, each flask containing 50 ml. of the buffered nutrient agar. Five flasks were included in each series. A Youden quinhydrone pH apparatus was used in making the pH determinations. The pH value of the cultures in each series is shown below:

SERIES	pH VALUE	SPECIFIC ACIDITY
I	4.28	525.0
II	5.94	11.5
III	6.21	6.17
IV	6.46	3.47
V	6.99	1.02
VI	7.51	0.309
VII	7.70	0.20
VIII	7.97	0.107

Funaria hygrometrica was the species used in these experiments. It was used by APINIS (1) and SCHWEIZER (6). Spores were germinated on the liquid nutrient solution in a Petri dish. After germination, and when the protonemata were long enough to be handled easily, protonemata from several spores were transferred to each flask by means of a fine glass needle.

The cultures were kept at room temperature and the source of illumination was light from a laboratory window.

Observations and discussion

Several studies have been made of the hydrogen-ion concentration of the substrate on which *Funaria hygrometrica* occurs in nature. MONTGOMERY (4) listed the occurrence of that species in a pH range of 5.2 to 7.6. His observations were based on two tests. IKENBERRY (2) found *Funaria hygro-*

metrica in a range of slightly less than pH 7 to pH 9, most frequently around pH 8. His conclusions were based upon 56 separate collections. APINIS (1) found that the same species grew in a pH range of 5.8 to 8.4, usually in pH 6.0 to 8.0. He also observed that though the spores germinated and protonemata developed in a pH range of 4.5 to 9.0, "Moss development is detected only on neutral and alkaline substrata pH 7.1 to 8.8." These pH values, indicating the range in which leafy plants were formed most abundantly, correspond closely to the pH value reported by IKENBERRY (2) for most frequent occurrence of that species (pH 8). They appear to support the conclusion of APINIS (1) that there is a correlation between the pH of the substrate where a species of moss occurs in nature and the pH of the artificial medium on which protonemata develop leafy plants.

The pH values of the culture series used in this investigation ranged from approximately 4 to 8. Some of these values are considerably more acid than those of the substrates on which *Funaria hygrometrica* has been found growing in nature. The pH values of series I through series V, pH 4.28 to 6.99, are much more acid than the neutral or alkaline substrates of pH 7.1 to 8.8 on which the development of leafy plants is reported to have been limited in the investigations of APINIS (1).

Final observations were made at the conclusion of a growing period of approximately three months. Protonemata grew and leafy plants developed in cultures at every pH value. These results indicate that protonemata of *Funaria hygrometrica* produce leafy plants on substrates that are considerably more acid in reaction than those on which that species usually occurs in nature. Furthermore, the pH values of the media on which leafy plants develop are much more acid than the limited range in which that development is reported by APINIS (1).

These observations support the general conclusion of SCHWEIZER (6) that leafy plants of *Funaria hygrometrica* develop on substrates of both high and low acidity though the cultures used were mostly in the acid range of pH values. No evidence has been found to support the experimental results of APINIS (1) or his suggestion relative to a possible relation between the pH of the substrate where a moss species occurs in nature and the pH range of the artificial media on which leafy plants develop.

Summary

Results of investigations with *Funaria hygrometrica* grown from primary protonemata on buffered nutrient agar substrates of pH values ranging from 4.28 to 7.97 indicate that there is no clearly defined correlation between the pH value of the substrate on which that moss species most frequently occurs in nature and the pH value of the artificial media on which leafy plants develop.

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LITERATURE CITED

1. APINIS, A. Data on the ecology of bryophytes III. The significance of hydrogen-ion concentration on germination of spores and development of some mosses. Acta Horti Bot. Univ. Lat. **11/12**: 1–22. 1939.

2. IKENBERRY, G. J. The relation of hydrogen-ion concentration to the growth and distribution of mosses. Amer. Jour. Bot. **23**: 271–279. 1936.

3. KESSLER, B. Beiträge zur Ökologie der Laubmoose. Beih. bot. Centralblatt. **31**: 358–387. 1914.

4. MONTGOMERY, C. E. Ecology of the mosses of the Grand de Tour region of Illinois, with special reference to hydrogen-ion relations. Bot. Gaz. **91**: 225–251. 1931.

5. PRINGSHEIM, E. G. Physiologische Studien an Moosen. 1 Mitt. Die Reinkultur von *Leptobryum piriforme* (L.) Schpr. Jahrb. wiss. Bot. **60**: 499–530. 1921.

6. SCHWEIZER, G. Physiologisch-morphologische Studien über *Funaria hygrometrica* L. in Reinkultur. Ber. d. bot. Ges. **48**: 75–84. 1930.

7. TREBOUX, O. Die Keimung der Moossporen in ihrer Beziehung zum Lichte. Ber. d. bot. Ges. **23**: 397–401. 1905.

BLOSSOM INDUCTION OF THE CRANBERRY

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(WITH TWO FIGURES)

As part of a study of the growth and fruiting habits of the cranberry (3) data were collected to determine the time of blossom induction. The method used was similar to that employed in finding the time of induction of the

FIG. 1. Cranberry blossom initiation, 1942 variety McFarlin. A. Tip of vegetative upright, June 19. B. Terminal bud, July 21. C. Blossom primordia, at cross, July 29. D. Blossom development, August 17. E. Same, September 8.

apple (4). Twenty-five to thirty uprights were defoliated (except the 2 or 3 terminal leaves) at 5- to 8-day intervals from the time the earliest blossoms hooked (turned down), about June 4, until three to four weeks after shoot growth was completed, on August 13.

An exact dating of induction is difficult because of the range of development which is typical of the cranberry. For example, the blossom period extended from June 10 until July 21 and the period of full blossom from June 25 to July 8. The growth of non-blossoming uprights was completed about July 20 as determined by averaging the lengths of 60 to 75 new growths weekly. Many new growths would have been through elongating 10 to 15 days previously.
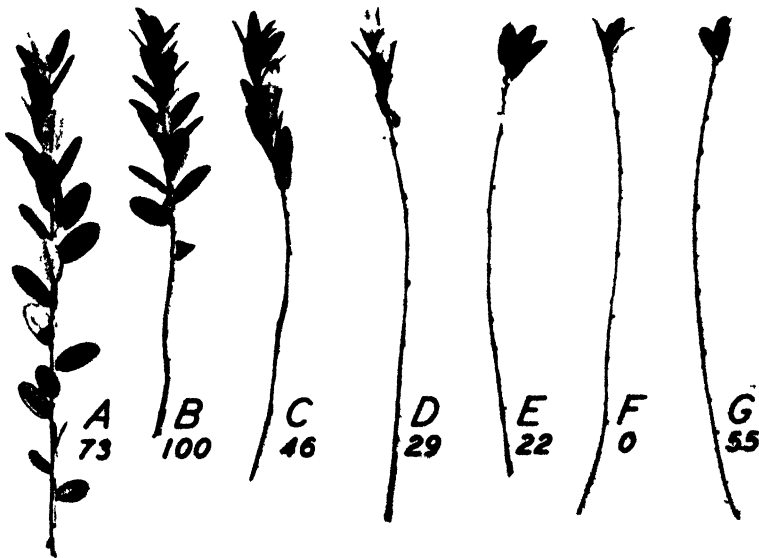
FIG. 2. Samples of uprights photographed September 16. Numbers show percentages of blossom buds formed. A. Untreated upright. B. Defoliated, June 4. Much new growth added. C. Defoliated, June 10. D. Defoliated, June 18. E. Defoliated, June 25. Some new leaves formed after defoliation. F. Defoliated, July 8. No new leaves. G. Defoliated, July 21. The presence of blossom buds on these shoots with no new leaves indicates induction was under way prior to defoliation.

The first blossom primordia on untreated uprights were found July 29 (fig. 1, C). This compares well with the observations of GOFF in 1901 (1) and those of LACROIX (2) on plants growing in Massachusetts.

The defoliated uprights were collected September 16 and the tips examined for blossom primordia. Representative shoots are shown in figure 2. Elongating growth and new leaves were formed following leaf removals until June 25. These new leaves induced blossom buds. Defoliation on July 8 inhibited bud formation (except on the few uprights upon which a

second growth and new leaves were produced following leaf removal). Many of the uprights which were defoliated July 15 differentiated blossom buds. That is, removal of the leaves on this date did not prevent bud formation. This is taken as evidence that induction was under way prior to this time. Uprights which were defoliated at later successive intervals showed increasing percentages of blossom buds. The dates of defoliation and percentages of blossoms formed follows (see fig. 2): June 4, 100.0; June 10, 45.5; June 18, 28.5; June 25, 21.5; June 30, 12.5; July 8, 0.0; July 15, 38.4; July 21, 54.5; July 29, 59.1; August 4, 72.7; and August 13, 80.0. Undefoliated uprights had 72.1 per cent. of terminal blossom buds. The reason for the lower percentage of blossom bud formation on the check uprights than on the earlier defoliated ones and on the last ones to be treated, was because the checks included some later-starting and less vegetative shoots than those selected for the defoliation tests. Only the more prominent, and so, more vigorous growths were used for leaf removals, particularly early in the season.

In 1942, induction of McFarlin cranberry blossom buds at Black River Falls, Wisconsin, was approximately July 10 and the earliest observed blossom primordia were found July 29, approximately three weeks later.

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LITERATURE CITED

1. GOFF, E. S. Investigation of flower-buds. Ann. Rpt. Wisconsin Agr. Exp. Sta. **18**: 304–319. 1901.
2. LACROIX, D. S. Cranberry flower-bud investigations. Jour. Agr. Res. **33**: 355–363. 1926.
3. ROBERTS, R. H., and STRUCKMEYER, B. ESTHER. Growth and fruiting of the cranberry. Proc. Amer. Soc. Hort. Sci. **40**: 373–379. 1942.
4. STRUCKMEYER, B. ESTHER, and ROBERTS, R. H. Investigation on the time of blossom induction in Wealthy Apple trees. Proc. Amer. Soc. Hort. Sci. **40**: 113–119. 1942.

NOTES

Annual Election.—The annual election of the American Society of Plant Physiologists has resulted in the naming of Dr. B. S. MEYER of The Ohio State University as president for 1943–1944. Dr. PAUL J. KRAMER, Duke University, is vice-president; and Dr. EARL S. JOHNSTON, The Smithsonian Institution, Washington, D. C., has been chosen secretary-treasurer. A tie vote for one of the offices will have to be decided by the executive committee.

Sectional Meetings.—One after the other, the regional sections have found it impossible to hold the meetings which would have been held in 1943. The Southern Section usually meets in February with the Southern Agricultural Workers, but the latter group held no meeting. The New England Section has its annual meeting in May, but postponed meeting again until conditions for travel are more favorable. The Western Section had planned a meeting at Corvallis, Oregon, but rationing and transportation problems led to the cancellation of the meeting. It seems probable now that no meetings can be held until the restrictions upon such activities are relaxed. In the meantime the American Society of Plant Physiologists and its Sections will find it necessary to transact business. The constitutional provisions should be followed strictly where provisions exist; and where they do not, steps should be taken to provide the necessary machinery for the transaction of business. The constitution of the parent society, By-Laws Section 2 a, provides that: "The executive committee shall have power to consider and act upon all matters not reserved to the Society as a whole." Steps should be taken at once by the executive committee to prescribe the methods to be employed during this meetingless interim. It would be unfortunate to allow irregularities to creep into our transaction of any required business.

On May 7, 1943, the American Society of Plant Physiologists lost one of its oldest members by death. Dr. ALEXANDER PIERCE ANDERSON of Red Wing, Minnesota, passed his eightieth birthday anniversary on November 22, 1942. He became a member of the Society not long after it was organized, his name appearing in the second membership list issued in the spring of 1927. He was a staunch supporter of the Society, having been one of the generous donors who helped to found the STEPHEN HALES award in 1927. Later on he became one of the small list of patrons (the second of them to be deceased), and in 1937 he was honored at Indianapolis by being awarded a CHARLES REID BARNES life membership.

A brief biography of Dr. ANDERSON was published in PLANT PHYSIOLOGY **13**: 214–215. 1937, so that it is not necessary to repeat the details of his life work. He was a fellow of the American Association for the Advance-

ment of Science, a member of the American Forestry Association, the Minnesota Academy of Sciences, the Minnesota Historical Society, and the Geological Society of Minnesota. His experimental work on cereal foods was very extensive, and embraced over 15,000 experiments.

To all surviving members of his family we extend our sympathy. If man's greatness is in proportion to the extent of his service to all, Dr. ANDERSON was truly a great man; for his work contributed to the pleasure and welfare of the whole world.

Tissue Culture.—An unusually fine work has come to the attention of plant physiologists in PHILIP R. WHITE's *A Handbook of Plant Tissue Culture*. It lives up to its name, as it is a small volume, a genuine handbook, and the subject is presented with a fulness and clarity that one seldom finds in a brief book. Dr. WHITE is a gifted writer, and it is a great service that he has rendered in the preparation of this work. There are ten chapters in all: The introduction, setting forth the problems of morphogenesis and the origin of functions; the history of plant tissue culture; the living materials; the laboratory; nutrients; how cultures are started; culture techniques; growth measurements and their interpretation; tissue culture and the study of problems in the pathology and general physiology of plants; and morphogenesis. A bibliography of 457 titles, and a general index complete the work. It is well illustrated, with 71 figures. One of the appreciated features is the series of portraits of the investigators who have contributed much to the field. The detailed description of the laboratory, and the meticulous directions for manipulation of apparatus, the making of media, recording results, interpreting and applying the techniques to new problems will be extremely helpful to anyone interested in doing work with tissue cultures. It is published by the Jaques Cattell Press, Lancaster, Pennsylvania, at $3.75 per copy. While the book has 276 pages, the text occupies but 226 pages. The print is large and clear, the pages small, and one can read all of it in a few hours. It should find a place in every physiologist's library.

· **Enzymes.**—A good book on enzymes for the student and investigator comes from the Cornell University Biochemistry Department. It is entitled *Chemistry and Methods of Enzymes,* and the authors are Dr. JAMES B. SUMNER and Dr. G. FRED SOMERS. It is an excellent general survey, without too much detail. It is divided into four parts, the first of which is an introductory chapter on the general properties of enzymes. Part 2 is devoted to the hydrolytic enzymes, seven chapters, as follows: Esterases; carbohydrases; enzymes of carbohydrate metabolism; nucleases; amidases; and proteolytic enzymes. Part 3 considers the oxidative enzymes. The chapter headings are: Oxidizing enzymes; the iron enzymes; the copper enzymes; dehydrogenases containing coenzymes 1 and 2; oxidases which transfer

hydrogen to cytochrome; the yellow enzymes; nuclein deaminases; miscellaneous oxidases; and desmolases.

Part four discusses the following: Hydrases and mutases; and carbohydrate metabolism. There are author and subject indexes, also a short history of enzymes down to 1931, and a literature list just preceding the introduction. The history is told in less than thirty events which were milestones of progress during about 150 years. Each chapter is closed by a list of pertinent references. The reviewer is very favorably impressed with the work, and it ought to help clarify what has become a very difficult field if all of the details are considered.

It is of modest size, only 365 pages, and the price per copy is $5.00. The publishers are Academic Press, Inc., 125 East 23rd St., New York, N. Y.

Vitamins and Hormones.—The first volume of an annual series on vitamins and hormones bears this title. The editors are Dr. ROBERT S. HARRIS and Dr. KENNETH V. THIMANN, of Harvard University. It bears a foreword by Dr. E. V. McCOLLUM of Johns Hopkins University. There are 10 reviews, with the following titles and authors: Choline—chemistry and significance as a dietary factor, by C. H. BEST and C. C. LUCAS; the appraisal of nutritional states, by NORMAN JOLLIFFE and RITA M. MOST; physical methods for the identification and assay of vitamins and hormones, by JOHN F. LOOFBOUROW; the chemical and physiological relationship between vitamins and amino acids, by H. H. MITCHELL; the photoreceptor function of the carotenoids and vitamins A, by GEORGE WALD; the significance of the vitamin content of tissues, by ROGER J. WILLIAMS; growth-factors for protozoa, by RICHARD P. HALL; physiology of anti-pernicious anemia material, by GEORGE R. MINOT and MAURICE B. STRAUSS; the intermediate metabolism of the sex hormones, by GREGORY PINCUS and WILLIAM H. PEARLMAN; and the hormones of the adrenal cortex, by T. REICHSTEIN and C. W. SHOPPEE.

The work constitutes a review of certain aspects of biochemistry. Such works have become a necessity to the busy investigator, and for those working on vitamins or hormones, or for those who desire technical information on the subjects covered. The value of the reviews depends upon the thoroughness with which the ground is covered, and the skill of the reviewers in summarizing and interpreting the trends of research. This volume seems to have been carefully prepared, and we hope that the editors and publishers succeed in establishing the series as an annual service.

This first volume contains 452 pages, and its list price is $6.50. The publishers are Academic Press, Inc., New York, N. Y.

Annual Review of Biochemistry.—Volume XII of this annual is of more than usual interest to the plant physiologist because of the large number of the reviews that fall more or less directly in the field of plant physiology. Naturally some of the reviews are in the animal field, but every physiologist finds it valuable to know something of both animal and plant

physiology. One cannot do justice to these fine reviews because of the number and variety of subjects covered. It is possible, however, to list them so that prospective purchasers will know the nature of the volume. There are 24 reviews, with the following titles and authors: Biological oxidations and reductions, by F. LIPMANN; proteolytic enzymes, by R. M. HERRIOTT; the steroids, by H. SOBOTKA and E. BLOCH; the chemistry of the proteins and amino acids, section I by L. F. HEWITT, and section II by R. A. KEKWICK and A. S. MCFARLANE; the chemistry and metabolism of the compounds of sulphur, by J. C. ANDREWS; carbohydrate metabolism, by H. J. DEUEL, JR.; fat metabolism, by G. O. BURR and R. H. BARNES; the metabolism of proteins and amino acids, by H. BORSOOK and J. W. DUBNOFF; the chemistry of the carbohydrates, by H. S. ISBELL; the chemistry of the lipins, by S. J. THANNHAUSER and G. SCHMIDT; mineral nutrition, by L. A. MAYNARD and J. K. LOOSLI; the chemistry of the hormones, by H. FRAENKEL-CONRAT; water-soluble vitamins, by R. J. WILLIAMS; fat-soluble vitamins, by K. HICKMAN; nutrition, 1941 and 1942, by C. S. LANGFORD and H. C. SHERMAN; animal pigments, by C. RIMINGTON; synthetic drugs, by T. C. DANIELS; photosynthesis, by E. S. JOHNSTON and J. E. MYERS; mineral nutrition of plants, by D. I. ARNON; carbon dioxide assimilation in heterotrophic organisms, by C. B. VAN NIEL; the electron microscope in biology, by L. MARTON; the chemistry of viruses, by C. L. HOAGLAND; and microchemistry, by A. A. BENEDETTI-PICHLER. The customary author and subject indexes close the volume.

This series of volumes has been successful, no doubt beyond all anticipation by the editors when they first projected the series. It has been growing in importance and value with the years, until now it constitutes the best source of information biochemists have aside from the original papers on which the reviews are based. The latter are frequently not available, and the reviews constitute an outstanding service to all investigators. If a worker in biochemistry could have but one series of volumes at his side, it should be a complete file of the Annual Review of Biochemistry.

The price is still only $5.00, and orders for it should go to Annual Reviews, Inc., Stanford University P.O., California.

Minor Elements.—The Fourth Supplement to the Third Edition of the well-known *Bibliography of References to the Literature on the Minor Elements and their Relation to Plant and Animal Nutrition* has been published by the Chilean Nitrate Educational Bureau, Inc., under the direction of HERBERT C. BREWER. It contains 92 pages, and is arranged in the same manner as previous supplements. More than half of the known elements are included, and some 900 or more authors have investigated the elements in connection with more than 100 crop plants.

The value of this annotated bibliography to plant physiologists and agricultural workers has been amply demonstrated. It is the best example of bibliographic service we have, and is highly appreciated by students and

investigators alike. For copies, write to HERBERT C. BREWER, Director, The Chilean Nitrate Educational Bureau, Inc., 120 Broadway, New York, N. Y.

Evaporation and Transpiration.—Publication no. 550 of the Carnegie Institution of Washington, Washington, D. C., is entitled: *Studies of Evaporation and Transpiration under Controlled Conditions.* The author is EMMETT MARTIN. It is a critical study of the quantitative aspects of evaporation and transpiration to test the adequacy of LEIGHLY's formula, which was found to be inadequate. Using blotting-paper evaporimeters of various sizes and shapes, MARTIN has found that the rate of evaporation per unit area of surface varied directly with the square root of the wind velocity, inversely with the 0.3 power of the dimension parallel to the wind flow, and inversely with the 0.2 power of the dimension at right angles to wind flow.

Studies were also made of the correlation between the rate of transpiration and relative humidity at three temperatures, 27°, 38°, and 49° C., with ample soil moisture. Young plants showed approximately linear relations except at 49°, at which temperature the rates at low relative humidities tended to run below the expected values. Older plants showed less transpiration at 27° than younger plants. At 38°, the rates were lower in older plants than young only when the humidities fell below 50 per cent. At 49°, the difference between old and young plants disappeared, presumably because of changes in the permeability of protoplasm conditioned by age and temperature.

The effects of wind on transpiration rates increased with temperature increase, probably because of increasing cuticular transpiration. Wind velocity of 250 cm./sec. at high temperatures and low relative humidities sometimes resulted in closure of stomata in less than 3 minutes.

The author studied the ratios of nighttime to daytime transpiration rates, the temperature depression of leaves (20° maximum depression), the effects of radiation on the transpiration rate, the importance of leaf size, and the rate of energy exchange under different conditions.

It is an interesting and valuable contribution to the dynamics of evaporation and transpiration, and the factors which modify plant behavior. Copies may be obtained from The Carnegie Institution of Washington, Washington, D. C.

Soil Science.—*The Fundamentals of Soil Science* is a text book on soil science by C. E. MILLAR and L. M. TURK, of the Michigan State College. It presents the basic principles of soil science in easily understood terms. It is practical, and broad. Other agricultural fields to which soil science contributes are brought into the discussion, and the general relations of soil to society are recognized.

The development is fairly logical. The first chapter deals with soil development, the second with soil classification, and the third with the physical and chemical properties of soils. Then the authors take up soil reaction,

lime and its uses, soil moisture, soil organisms, and soil organic matter. Following these chapters, cover and green manure crops, farm manures, and the nutrient requirements of plants are considered, and the soil amendments, fertilizers, fertilizer practices, and the maintenance of soil fertility and productivity of the soil. The remainder of the chapters are: Soils and agriculture of arid regions; irrigation; fruit soils; lawn soils; and soil resources. The reviewer considers it a very good survey of soil science at the present time, and recommends it to students and instructors. It is a considerable improvement over many an older text.

The publishers are John Wiley and Sons, Inc., New York, N. Y., who quote the price of the volume at $3.75. With index it contains 462 pages; it is illustrated with 78 figures. A glossary is provided for those who are unfamiliar with the terms used.

Annual Review of Physiology.—The fifth volume of the *Annual Review of Physiology* contains 24 reviews, two of which are in two parts, so that there are really 26. They deal almost exclusively with animal physiology, although such reviews as those by C. V. TAYLOR on the physical aspects of protoplasm, and by J. SCHULTZ on physiological aspects of genetics are certainly of more general interest. In addition to these topics, we note reviews dealing with the physiological and pathological aspects of ultraviolet radiation; developmental physiology; physiology of bone; energy metabolism; the respiratory system; muscle; the digestive system; blood; the lymphatic system; heart; nerve and synaptic transmission; visceral functions of the nervous system; temperature regulation; liver and bile; sense organs, (I) vision; (II) special senses other than vision; metabolic functions of the endocrine system; physiology of mammalian semen; endocrinology of reproduction; physiological psychology, (I) the functional psychoses; (II) physiological correlates of behavior; and biological assay. There is an author index, and a subject index, as usual.

The reviews are prepared by experts in the respective fields, and under the present disturbed world conditions it is remarkable that the authors can do as well as they do. This annual review is serving the field of physiology admirably, and it deserves the support of those who need the summaries, and that includes the physiological and medical fraternity generally.

The price of the *Annual Review of Physiology* is $5.00 per copy. It contains 612 pages, and copies may be ordered from Annual Reviews, Inc., Stanford University P.O., California.

PLANT PHYSIOLOGY

OCTOBER, 1943

A NEW TYPE OF INTERMITTENTLY-IRRIGATED SAND CULTURE EQUIPMENT[1]

Hugh G. Gauch and Cecil H. Wadleigh

(WITH TWO FIGURES)

A type of sand culture apparatus with automatic flushing suitable for the growth of small plants in the greenhouse has recently been described by Eaton.[2] This apparatus fulfills certain conditions necessary in the culture of plants on saline nutrient substrates. By means of frequent irrigations

FIG. 1. General view of apparatus with plants.

with an excess of solution, the salt content of the solution in the root zone does not become unduly concentrated as a result of continued water absorption and of evaporation from the surface of the sand.

The present discussion deals with a similar type of greenhouse sand culture equipment of larger dimensions—five times the volume of sand and

twice the volume of nutrient solution—and possessing the advantage that most of the necessary equipment is readily available or easily obtainable under normal conditions. This apparatus has proved successful for growing strawberry and Ladino clovers, alfalfa (fig. 1), beans, flax, and guayule. The size of the sand crock and solution reservoir is amply sufficient, however, for the growth of a mature tomato plant. Very little manual attention is required, and the apparatus has proved relatively trouble-free operation.

The method of irrigation in this apparatus is very similar to the various large-scale beds used for commercial or research purposes. difficulty with such beds, however, is that for research purposes the bed ; the statistical unit. Replication is limited, and therefore there is not t¹ degree of statistical precision obtainable with the individual culture units herein described.

Description and operation of apparatus

Each unit is composed of the following main items: A 5-gallon glazed crock (34)³ filled with sand (35)⁴ in which the plant or plants are grown and a 5-gallon glazed crock (21) to serve as the solution reservoir; a half-gallon glass jug (14) which delivers two quarts of nutrient solution to the plants at each irrigation; a check valve (24) to control the direction of flow of solutions; and a compressed air-line (5).

At hourly, or other selected intervals, compressed air is delivered to the glass jug forcing the solution up through the effluent tube (19, 33) to the top of the sand culture and out through the holes in the aluminum ring (29). This pressure on the solution in the jug and in the refill tube (13) forces the glass bead (25) to the bottom of the valve (24) where it forms a seal against the rubber tubing (26) and prevents the escape of solution back into the reservoir (21) during the irrigation. After a lapse of time sufficient for emptying the half-gallon bottle (approximately 2 minutes), the solenoid valve in the air supply line closes, and simultaneously another solenoid valve on the end of a "T" in the system air-line opens, thus relieving the pressure in the air-line (5). At this time solution flows by force of gravity from the reservoir to refill the jug.

The excess of solution delivered to the sand culture returns through a drain tube (18) to the reservoir (21).

Each unit has 26 liters of solution of which approximately 6 liters are retained by the sand, 2 liters in the jug, and approximately 18 liters in the reservoir. By noting the original level of solution in the reservoir, the volume of solution is maintained by the addition of distilled water.

³ Numbers in parentheses refer to number-labels in figure 2.

⁴ The sand should be of a degree of fineness which will prevent too rapid a rate of percolation of water through the sand, since it is desirable to have a sheet of water developed over the surface of the sand at each irrigation. Sand of the following composition has given satisfactory results in our work: not less than 85 per cent. by weight to pass a 20-mesh screen and be retained on a 100-mesh screen.

Description of parts shown in figure 2

A. Glass bead check valve.

    1. Rubber tubing sealed to the glass tube (2) with Automotive Bond Cement. The lower end of the rubber tubing is serrated to prevent formation of a seal when the glass bead rises to the top of the valve during refilling of the jug.

    2. 10-mm. O.D. glass tubing, $3\frac{1}{2}$ inches in length.

    3. Glass bead, 4 mm. in diameter.

    4. Rubber tubing sealed to the glass tubing (2) with Automotive Bond Cement. By means of a hot, metal ball bearing, a seat for the glass bead is seared into the upper end of this piece of rubber tubing. After cooling, the excess melted rubber may be removed by use of benzine or other suitable solvent, and then the surface should be thoroughly dusted with talc.

B. Sand culture apparatus.

    5. $\frac{3}{4}''$ galvanized iron pipe which serves as the air-supply line for each group of 10 cultures (see also fig. 1).

    6. $\frac{1}{4}''$ copper tubing outlet from main air-line pipe.

    7, 9, 11, 22, 27, 32, 36. Rubber tubing connections.

    8. Two-inch piece of $\frac{1}{2}$-mm. capillary tubing, selected for uniformity, to serve as a modulator of the air-supply, and thereby ensure uniform air-pressure to all cultures.

    10. 3-mm. O.D. glass tubing.

    12. Wooden plank, $1'' \times 14'' \times 8\frac{1}{2}'$, with $2\frac{1}{2}''$ holes approximately 20 inches apart.

    13, 18, 19, 33. 5-mm. O.D. glass tubing.

    14. One-half gallon glass jug.

    15. No. 6 long-taper rubber stopper. (This stopper should be anchored in place by a light wire brace extending over stopper and threaded through the handle of the jug.)

    16, 39. No. 3 rubber stopper.

    17. Wooden skid, $2'' \times 2'' \times 8\frac{1}{2}'$.

    20. Culture solution.

    21, 34. 5-gallon glazed crock.

    23, 24, 25, 26. "A–1, –2, –3, –4," respectively.

    29. Soft aluminum tubing ($\frac{3}{16}''$) to form a complete ring with the exception of the arms of the glass T-tube (31) and the rubber tubing connections.

    30. $1/32''$ outlet hole in the tube. These holes should be spaced about $1\frac{1}{2}''$ apart on the inner surface of the ring (i.e., toward center of crock) and slightly below center so that the solution will be directed downward at about a 45° angle.

    31. Glass T-tube.

    35. Fine quartz sand (see footnote no. 4).

37. Quartz rock.

38. Wire screen or gauze, 2″ × 2″, with a notch or a hole to accommodate the upright solution-delivery tube (33).

Substitutions: For all "rubber tubing connections" (*i.e.*, 7, 9, 11, 22, 27, 32, 36) windshield wiper hose (cloth inserted rubber) may be substituted. Additional features of this type of hose: Heat resistant; oil resistant; r forced side walls; kink-proof; and long life.

Saran tubing may be substituted for the soft, aluminum tubing (2 `
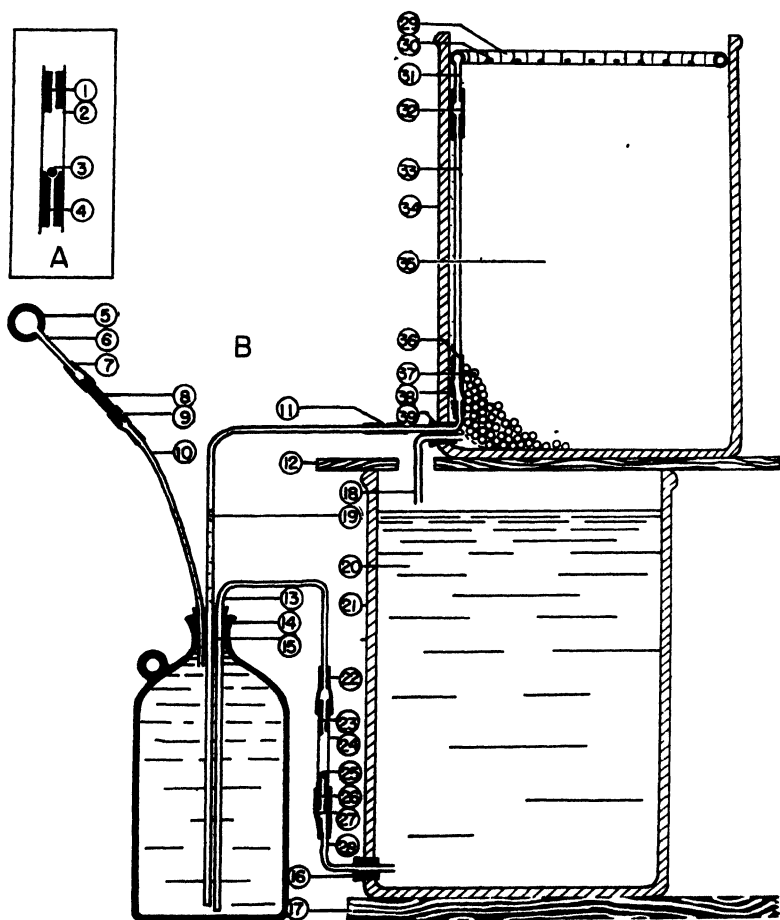
FIG. 2.   Cross section view of sand culture apparatus.   (Description in text.)

Source and control of the compressed air

For operating 100 cultures by this method, one should have a source of compressed air which delivers 6 cu. ft. of air per minute at 5 to 7 lb. pressure per square inch.   If a source of compressed air is already available but the pressure is in excess of 5 to 7 lb. per square inch, a pressure-control diaphragm valve which will reduce the pressure to the desired level is necessary.

Time switches regulate the time at which this air-delivery valve opens and the length of time it remains open. The following time switches are suitable:

1. AN INTERVAL TIME SWITCH.—An interval time switch can be used alone without a master switch (2, below) if the cultures are to be irrigated each hour throughout a 24-hour period. This type of timer makes a complete ꞏꞏꞏꞏle every 60 minutes. The duration of the "on" period can be set for any ꞏꞏꞏtion of the 60-minute period. In this switch two circuits are involved— one being "on" while the other is simultaneously "off," and vice versa. ꞏꞏ compressed air is permitted to flow into the line for three minutes ꞏꞏ the "on" period of one circuit, then subsequently the solenoid valve which reduces the pressure to atmospheric pressure will be activated and held open for the remainder of the hour (57 minutes) by the other circuit.

2. MASTER SWITCH.—This switch is connected with the interval time switch (1, above) in such a way that the circuit to the solenoid valve controlling the air supply is completed only during the "on" period of both time switches. The "on" and "off" pins on the 24-hour dial of the master switch cannot be inserted nearer together than every 15 minutes. This switch is thus used only to limit the irrigations to selected hours, and the interval time switch determines the length of time during which there will be compressed air in the air-line (5).

If there is no present available source of compressed air, a small diaphragm compressor may be used; then the time switches control the motor on the compressor instead of the solenoid valve on a main air-supply line.

Solution change or solution renewal

Certain small diaphragm compressors, such as are used for paint-spraying outfits, may be used to produce a vacuum instead of a pressure by fixing a hose connection on the air-intake. This vacuum may be conducted to a 5-gallon carboy and a second hose from the carboy used to remove the 18 liters of solution in the reservoir.

Control of algal growth

All external glass tubing (except the valve) should be painted black. It is preferable to wrap the valve with black friction tape so that one may easily check on the functioning of the valve. A heavy paper bag, sprayed inside and out with black paint, should be tied around the glass jug (see fig. 1).

After the seedlings have attained sufficient size, a one-half to one-inch layer of quartz rock may be added over the surface of the sand. These larger particles dry thoroughly between irrigations and the growth of algae on the surface is thus prevented or greatly inhibited.

In order to inhibit the growth of algae in the reservoir, a covering is laid over the hole in the plank through which the drain tube (18) passes (see fig. 1).

U. S. REGIONAL SALINITY LABORATORY
RIVERSIDE, CALIFORNIA

A BIOCHEMICAL STUDY OF CURING PROCESSES
IN SWEET POTATOES[1]

PETER H. HEINZE AND C. O. APPLEMAN

Introduction

If sweet potatoes are to be stored for any length of time they ι
subjected immediately after digging to a suitable combination of tι
ture and humidity for effective curing of the roots. The physiology of
ing is imperfectly understood because of our limited information of the
essential chemical changes in the roots during curing. Previous studies
have been confined chiefly to carbohydrate transformations and respiration.
The rate of respiration decreases over that of freshly harvested potatoes
(1, 5). Both sucrose and reducing sugars accumulate at the expense of the
starch (1, 3, 4). The percentage of moisture changes very little regardle, s
of the amount of shrinkage by loss of water (3, 5). The pH data for ex-
pressed juice do not give any significant information on the curing process
(5).

The present investigation has been concerned with the nitrogen metab-
olism and pectic transformations in Maryland Golden Sweet Potatoes dur-
ing curing under different combinations of temperature and humidity and
also during subsequent storage under favorable conditions. The keeping
qualities of the sweet potatoes cured under the different conditions were
recorded in order to determine the relationship of the nitrogen metabolism
and pectic changes in the roots during curing to their keeping qualities in
storage. The results are also of general physiological interest in relation to
the effect of temperature and humidity on nitrogen distribution in fleshy
roots.

The sweet potatoes for the experimental lots were carefully gathered in
the field and placed under experimental conditions the day following their
harvest. The lots were cured for 10 or 11 days under the different com-
binations of temperature and humidity indicated in the tables. All of the
lots at the end of the curing period were stored for four months in an elec-
tric refrigerator especially equipped to give the desired temperature con-
trol. All the lots were stored at 50° to 53° F. and 50 to 60 per cent. relative
humidity. Analyses were made at intervals during the curing and storage
periods.

Analytical methods

SAMPLING AND DETERMINATION OF MOISTURE.—Six representative sweet
potatoes were ground in a Nixtamal mill, and after thorough mixing,
samples of the fresh pulp were weighed out for moisture, pectins, and nitrog-

enous constituents.   To determine moisture, about 5 gm. of pulp were dried to constant weight at 80° C. in a vacuum of 30 inches of mercury.

TOTAL PECTIN.—Duplicate samples of 50 gm. of the freshly ground pulp were weighed into counterpoised Erlenmeyer flasks and covered with 260 l. of hot 95 per cent. alcohol.   After the storage alcohol had been removed ₁ filtration the samples were washed with alcohol and ether, dried at 60° C. a vacuum oven, and ground to pass a 60-mesh sieve.   A 2-gm. sample of tne dᵣⁱ ₁ material was placed in a 500-ml. Erlenmeyer flask, covered with of 0.5 per cent. ammonium citrate solution, and boiled very gently minutes.   The solutions were filtered into a 500-ml. volumetric flask. .e pulp was washed with warm water, returned to the Erlenmeyer flask with 100 ml. of N/30 hydrochloric acid, and refluxed gently for one hour. The material was again filtered and washed with warm water.   The filtrates were combined, made to volume, and the pectic materials precipitated from an aliquot as calcium pectate as described by CARRÉ-HAYNES (2).   The c· lcium pectate gels were found to contain impurities which were largely ɾoteinaceous.   The amount of impurities was estimated by determining the nitrogen content of a portion of the dried precipitate by the micro-Kjeldahl method and by calculating the protein content by use of the usual conversion factor, 6.25.   This value was subtracted from the weight of the original precipitate to give the amount of calcium pectate.

SOLUBLE PECTIN.—Four grams of the dried plant sample were transferred to a one-liter bottle with 500 ml. of 0.2 per cent. ammonium citrate solution.   After shaking for one hour on a mechanical shaker the solution was filtered and a 200-ml. aliquot taken for the determination of the soluble pectic materials, which were determined as described for total pectic material.

PROTOPECTIN.—The percentage of protopectin was calculated by subtracting the percentage of soluble pectin from the percentage of total pectin.

TOTAL NITROGEN.—Portions of the fresh pulp of approximately 5 gm. each were used for total nitrogen by the usual Kjeldahl method.   Since no nitrates could be detected the modification to include nitrates was omitted.

EXTRACTION OF NON-PROTEIN NITROGEN.—A sample of 100 gm. of the freshly ground pulp was placed in a mortar and a small quantity of acid washed quartz sand added.   The pulp was thoroughly triturated and after the gradual addition of 20 ml. of water the mixture was transferred onto a square of huck toweling suspended over a 2-liter beaker and the extract expressed by hand.   The residue was returned to the mortar and the extraction repeated two additional times.   The extract was centrifuged to remove as much starch as possible.   It was heated to boiling, a few ml. of 5 per cent. Fe_2O_3 solution were added, and the boiling continued for approximately 2 minutes.   The solution was filtered in a Buchner funnel containing an asbestos mat.   The beaker and the mat were thoroughly washed with hot water.   The filtrate was made to a volume of 1000 ml. and preserved with toluene when not analyzed immediately.

TOTAL NON-PROTEIN NITROGEN.—Aliquots of the non-protein filtr... analyzed as described for total nitrogen.

TOTAL ALPHA-AMINO NITROGEN.—A special preliminary procedure... fected by STUART and APPLEMAN (7) was adopted to eliminate errp... occur in the usual Van Slyke procedure. Aliquots of 10 ml. each... for the determination of total alpha-amino nitrogen in the Van Sly ratus. The volume of the gaseous nitrogen was reduced to stano... tions and the proper corrections made for the blank determinations.

BASIC NITROGEN.—Aliquots of 100 ml. of the non-protein extr... acidified with 2.5 ml. of concentrated sulphuric acid. When cooled temperature, 30 ml. of phosphotungstic acid solution (20 gm. ... tungstic acid and 5 gm. of sulphuric acid made to 100 ml. of solut... added. After standing for 24 hours in a refrigerator, the soluti filtered and the precipitates washed thoroughly with a dilute soln... phosphotungstic acid, containing 2.5 gm. of phosphotungstic acid and ... of sulphuric acid per 100 ml. of solution. The filter paper and the preci... tates were transferred to a Kjeldahl flask and digested as for total nitrog... The ammonia was distilled into 0.01 N acid and the excess acid titrated with 0.01 N base.

NON-AMINO NITROGEN.—The filtrates from the basic nitrogen samples were neutralized with concentrated sodium hydroxide. The solution was then subjected to the distillation treatment and the mono-amino nitrogen was determined in the Van Slyke apparatus.

AMIDE NITROGEN.—Aliquots of 50 ml. of the non-protein extract were hydrolyzed under reflux condensers with 3 ml. of concentrated sulphuric acid in a boiling water bath for 2.5 hours. The solutions were filtrated, the residue washed with a few ml. of dilute sulphuric acid, and the filtrate neutralized with sodium hydroxide. The ammonia was determined by the SESSIONS and SHIVE method (6).

HUMIN NITROGEN.—The residues filtered off following the amide hydrolysis were placed in a Kjeldahl flask and digested as for total nitrogen and the ammonia distillation was carried out as for basic nitrogen.

RESIDUAL NITROGEN.—The residual nitrogen was determined by finding the difference between the total non-protein nitrogen and the sum of the determined soluble nitrogen fractions. In the lots cured at 86° and 95° F. with low humidity, the residual nitrogen represents the difference between the total non-protein nitrogen and the sum of the total alpha-amino, amide, and ammonia nitrogen. In all other lots it represents the difference between the total non-protein nitrogen and the sum of the mono-amino, amide, basic, ammonia, and humin nitrogen.

Since there was considerable loss in total weight of sweet potatoes during curing and storage, all of the results are presented as percentages of the fresh weight at the time of harvest in order to have the percentages reveal actual changes in the constituents determined.

Results

CHANGES IN PECTIC SUBSTANCES

changes in the pectic substances during curing and storage are
:n table I.

TABLE I

THE PECTIC CONSTITUENTS IN SWEET POTATOES DURING CURING AND STORAGE.
THE RESULTS ARE EXPRESSED AS PERCENTAGES OF FRESH WEIGHT
AT HARVEST TIME

ΛΕΝΤ	DATE OF ANALYSIS	SOLUBLE PECTIN	PROTOPECTIN
Cured at 86° F. and low humidity			
		%	%
period	Oct. 12	0.399	0.409
	Oct. 22	0.326	0.532
Cured at 86° F. and 80 to 85% humidity			
Curing period	Oct. 7	0.426	0.350
	Oct. 13	0.428	0.375
	Oct. 18	0.574	0.302
Storage period	Nov. 24	0.535	0.411
	Jan. 1	0.298	0.639
	Feb. 22	0.339	0.654
Cured at 86° F. and 95 to 100% humidity			
Curing period	Oct. 7	0.426	0.350
	Oct. 18	0.538	0.309
Storage period	Nov. 24	0.465	0.548
	Feb. 22	0.372	0.717
Cured at 95° F. and low humidity			
Curing period	Oct. 12	0.399	0.409
	Oct. 22	0.425	0.551
Cured at 104° F. and 70 to 75% humidity			
Curing period	Oct. 7	0.426	0.350
	Oct. 13	0.445	0.465
	Oct. 18	0.582	0.294
Storage period	Nov. 24	0.458	0.377
	Jan. 1	0.252	0.670
	Feb. 22	0.302	0.759
Cured at 104° F. and 90 to 95% humidity			
Curing period	Oct. 7	0.426	0.350
	Oct. 13	0.442	0.414
	Oct. 18	0.598	0.255
Storage period	Nov. 24	0.483	0.554

In all of the lots except the one cured at 86° F. and low humidity, the
percentage of soluble pectin in the sweet potatoes increased during curing.
The protopectin showed a corresponding decrease. The rate of this trans-
formation was somewhat greater at the higher temperatures. At the stor-
age temperature the protopectin increased again while the pectin decreased.
Some of the increase in protopectin was due to an actual increase in the total
pectic substances. This storage synthesis of protopectin continued in the
roots as long as they remained alive and sound.

NITROGEN METABOLISM

In table II are shown the changes in the nitrogenous fractions in sweet potatoes during curing and storage. At the time of harvest the total

TABLE II

CHANGES IN THE DISTRIBUTION OF NITROGEN IN SWEET POTATOES DURING CURI' STORAGE. RESULTS ARE EXPRESSED AS PERCENTAGES OF FRESH WEIGHT AT HARVEST TIME

TREAT-MENT	DATE OF ANALYSIS	TOTAL N	PRO-TEIN N	NON-PRO-TEIN .N	ALPHA AMINO N	AMIDE N	BA ?
		%	%	%	%	%	%	%
				Cured at 86° F. and low humidity			
Curing period	Oct. 12	0.217	0.145	0.072	0.047	0.008	0.010	0.016
	Oct. 22	0.223	0.139	0.084	0.044	0.006	0.014	0.032
Storage period	Nov. 23	0.235	0.148	0.087	0.028	0.009	0.020	0.04''
	Dec. 31	0.210	0.131	0.079	0.024	0.015	0.017	0.0''
				Cured at 86° F. and 80 to 85% humidity			
Curing period	Oct. 7	0.190	0.149	0.041	0.029	0.0015	0.008	0.012
	Oct. 13	0.187	0.143	0.044	0.041	0.0021	0.009	0.011
	Oct. 18	0.162	0.116	0.046	0.025	0.0033	0.008	0.017
Storage period	Nov. 24	0.157	0.102	0.055	0.030	0.0029	0.014	0.015
	Jan. 1	0.145	0.098	0.047	0.026	0.0025	0.016	0.008
	Feb. 22	0.177	0.099	0.078	0.043	0.0063	0.016	0.026
				Cured at 86° F. and 95 to 100% humidity			
Curing period	Oct. 7	0.190	0.149	0.041	0.029	0.0015	0.008	0.012
	Oct. 18	0.189	0.132	0.057	0.030	0.0036	0.013	0.020
Storage period	Nov. 24	0.184	0.127	0.057	0.034	0.0040	0.011	0.011
	Feb. 22	0.162	0.113	0.049	0.026	0.0026	0.024	0.002
				Cured at 95° F. and low humidity			
Curing period	Oct. 12	0.217	0.145	0.072	0.047	0.008	0.010	0.016
	Oct. 22	0.250	0.150	0.100	0.061	0.002	0.022	0.036
Storage period	Dec. 10	0.207	0.113	0.094	0.025	0.016	0.017	0.051
	Feb. 10	0.208	0.118	0.120	0.013	0.025	0.022	0.090
				Cured at 104° F. and 70 to 75% humidity			
Curing period	Oct. 7	0.190	0.149	0.041	0.029	0.0015	0.008	0.012
	Oct. 13	0.159	0.095	0.064	0.049	0.0049	0.008	0.013
	Oct. 18	0.175	0.088	0.087	0.058	0.0086	0.013	0.019
Storage period	Nov. 24	0.185	0.109	0.076	0.040	0.0117	0.015	0.017
	Jan. 1	0.176	0.089	0.087	0.048	0.0071	0.022	0.013
	Feb. 22	0.178	0.083	0.095	0.049	0.0158	0.020	0.022
				Cured at 104° F. and 90 to 95% humidity			
Curing period	Oct. 7	0.190	0.149	0.041	0.029	0.0015	0.008	0.012
	Oct. 18	0.141	0.056	0.085	0.155	0.0084	0.013	0.018
Storage period	Nov. 24	0.164	0.091	0.073	0.041	0.0089	0.013	0.018
	Feb. 22	0.170	0.057	0.103	0.051	0.0169	0.020	0.022

organic nitrogen in the roots averaged only 0.2 per cent. of the fresh weight, or 0.8 per cent. of the total solids. The non-protein nitrogen in the roots at harvest time in percentage of total nitrogen was 21.58 per cent. .in one

op and 33.18 in another crop.   The non-protein nitrogen increased in all
ts during curing.   At the same time the protein nitrogen decreased, indi-
ating protein hydrolysis during curing.   The rate of hydrolysis was some-
higher at the higher curing temperatures.   There were consistent
es in the basic and residual nitrogen at all curing temperatures, but
at increases in the amino and amide nitrogen occurred only at high
emperature.
nitrogen distribution in the sweet potatoes during storage at 50°
remained fairly stable during most of the storage period but there
tite evidence of slight protein hydrolysis near the end of the storage
, especially in the lots cured at the highest temperature.   The slight
s in the amino nitrogen during the entire storage period are of doubt-

TABLE III

DISTRIBUTION OF NITROGEN IN THE PROXIMAL AND DISTAL HALVES OF SWEET POTATOES.
RESULTS ARE EXPRESSED AS PERCENTAGES OF THE FRESH WEIGHT
AT THE TIME OF ANALYSIS

	MOISTURE	TOTAL N	NON-PROTEIN N	ALPHA AMINO N	AMIDE N	BASIC N	RESIDUAL N
HARVEST SAMPLE, OCTOBER 7							
	%	%	%	%	%	%	%
Proximal half	75.68	0.187	0.044	0.020	0.003	0.010	0.017
Distal half	75.60	0.181	0.044	0.019	0.003	0.010	0.018
Potatoes cured and stored until November 27							
Proximal half	75.84	0.178	0.062	0.036	0.008	0.010	0.025
Distal half	75.81	0.172	0.054	0.032	0.007	0.013	0.013

ful significance but the amide, basic, and residual nitrogens showed signifi-
cant increases in most lots near the end of the storage period.   These nitro-
gen fractions were also calculated as percentages of total nitrogen and these
percentages definitely confirm the changes indicated in table II.   The
ammonia and humin nitrogens were determined but the results are not
included in the tables as the amounts were very small and they showed no
definite trends during curing or storage.   The mono-amino acid nitrogen
is not included in the tables as the changes in this fraction correspond almost
exactly with those of the alpha amino acid, the absolute quantities are less
by the alpha amino nitrogen in the basic fraction.

DISTRIBUTION OF NITROGENOUS COMPOUNDS IN THE PROXIMAL AND DISTAL
ENDS OF THE SWEET POTATO

Analyses were made of the proximal and distal halves of the harvest
sample and of potatoes that had been in storage for six weeks.   The samples

were extracted on the dates indicated in the table. The non-protein nitrogen solution was preserved with toluene, stored at room temperature, and analyzed on February 9. The results are given in table III.

The proximal half showed a slightly higher total nitrogen content. The variations were so small that it may be concluded there is no apparent dif* ence in the nitrogen distribution in the proximal and distal halve of sweet potato.

Summary

A study was made of the nitrogen metabolism and the pectic transfor· .-tions in Maryland Golden Sweet Potatoes during curing under differen' combinations of temperature and ·humidity and also during subsequ storage.

The soluble pectin in the sweet potatoes increased during curing. Tl protopectin showed a corresponding decrease. An increase in the total pectic material occurred in all lots.

At the storage temperature the protopectin increased again while the pectin decreased. Some of the increase in protopectin was due to an actual increase of the total pectic substances. This storage synthesis of protopectin continued in the roots as long as they remained alive and sound.

The non-protein nitrogen in the sweet potatoes at harvest time ranged from 22 to 33 per cent. of the total nitrogen. Under all of the curing conditions the non-protein nitrogen increased and the protein nitrogen decreased indicating protein hydrolysis. The rate of this hydrolysis was somewhat higher at the higher curing temperatures. Fairly consistent increases in basic, amide, and residual nitrogen occurred in all lots but a consistent increase in amino nitrogen occurred only in those lots cured at the high temperatures of 95° and 104° F.

The nitrogen distribution in the sweet potatoes during storage at 50° to 53° F. remained fairly stable during most of the storage period. The amide, basic, and residual nitrogens increased slightly in most lots near the end of the storage period.

There was no apparent difference in the nitrogen distribution in the proximal and distal halves of the Maryland Golden Sweet Potato.

A temperature of 86° F. for curing the Maryland Golden Sweet Potato, gave the best results in storage. Higher temperatures were unsuitable because of internal break-down during curing. At 86° F. a high relative humidity was the decisive factor for effective curing of this variety, but there was no conclusive evidence that humidity had any effect on the nitrogen metabolism in the roots during curing.

The curing of sweet potatoes appears to involve a number of interrelated internal processes. Studies to date do not reveal any one outstanding essential change in the potatoes during curing.

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LITERATURE CITED

1. APPLEMAN, C. O., and SMITH, C. L.   Effect of previous cold storage on the respiration of vegetables at higher temperatures.   Jour. Agr. Res. **53**: 557–580.   1937.

   'ARRÉ, M. H., and HAYNES, D.   The estimation of pectin as calcium pectate and the application of this method to the determination of soluble pectin in apples.   Biochem. Jour. **16**: 60–69.   1922.

   HASSELBRING, HEINRICH, and HAWKINS, L. A.   Physiological changes in sweet potatoes during storage.   Jour. Agr. Res. **3**: 331–342.   1915.

4. ——————, and HAWKINS, L. A.   Carbohydrate transformations in sweet potatoes.   Jour. Agr. Res. **5**: 543–560.   1915.

   JOHNSTONE, GEORGE R.   Physiological study of two varieties of *Ipomoea batatus*.   Bot. Gaz. **80**: 145–165.   1925.

6. SESSIONS, A. C., and SHIVE, J. W.   A method for the determination of inorganic nitrogen in plant extracts.   Plant Physiol. **3**: 499–511.   1928.

7. STUART, N. W., and APPLEMAN, C. O.   Nitrogenous metabolism in Irish potatoes during storage.   Maryland Agr. Exp. Sta. Bull. 372.   1935.

SOME EFFECTS OF SODIUM SALTS ON THE GROWTH OF THE TOMATO[1]

H. E. HAYWARD AND E. M. LONG

(WITH SIX FIGURES)

Introduction

In a previous study (6) the influence of high osmotic concentratic⊤ sodium salts and nutrient solutions upon the vegetative development (tomato was reported, but no data were obtained on the fruiting resp To investigate this problem, to obtain further information on the relative t⸱ icity of the Cl⁻ and SO₄⁼ ions, and the effect of osmotic concentration, exp⸱ ments were set up to determine the response of tomato plants to soc chloride and sodium sulphate when supplied together in different propoɪ tions and at several levels of total concentration.

Experimental procedure and methods

Marglobe tomatoes were grown under greenhouse conditions in automati-cally irrigated sand cultures of the type designed by EATON (2). Two series of five treatments each were set up with three replications. The treatments were: Base nutrient solution (control); and base nutrient ⸱ 40, 80, 120, and

TABLE I

CONSTITUTION OF CULTURE SOLUTIONS

SERIES AND CULTURE	TREAT-MENT	OSMOTIC CONC.	SALTS					
			$Ca(NO_3)_2$	KNO_3	$MgSO_4$	KH_2PO_4	NaCl	Na_2SO_4
	m.e. Na/l.	atm.	m.e./l.	m.e./l.	m.e./l.	m.e./l.	m.e./l.	m.e./l.
A & B-1	Control	1.6	14.4	14.4	14.4	1.8		
A-2	40	2.9	"	"	"	"	10	30
A-3	80	4.1	"	"	"	"	20	60
A-4	120	5.3	"	"	"	"	30	90
A-5	160	6.4	"	"	"	"	40	120
B-2	40	3.2	"	"	"	"	30	10
B-3	80	4.6	"	"	"	"	60	20
B-4	120	6.1	"	"	"	"	90	30
B-5	160	7.7	"	"	"	"	120	40

Micro-nutrients: B, 1 p.p.m.; Zn, 0.1 p.p.m.; Mo, 0.1 p.p.m.; Mn, 0.4 p.p.m.; Cu, 0.01 p.p.m.; Fe, 5 p.p.m., supplied as iron citrate.

Analysis of Riverside tap water, m.e./l.

Ca	Mg	Na	K	Cl	SO₄	HCO₃
1.40	0.23	1.51	0.05	0.45	0.75	2.50

ι60 milliequivalents per liter of sodium salts. To segregate the supplemen-
tary effects of the anions, 25 per cent. of the sodium was supplied as Cl⁻ and
5 per cent. as SO₄⁻ in series A; and 75 per cent. as Cl⁻ and 25 per cent. as
ᵗO₄⁻ in series B. The constituents of the base nutrient solution, the amounts
ᵈium salts added, the osmotic concentration of the culture solutions, and
ᵧsis of the tap water at Riverside, California, are shown in table I.
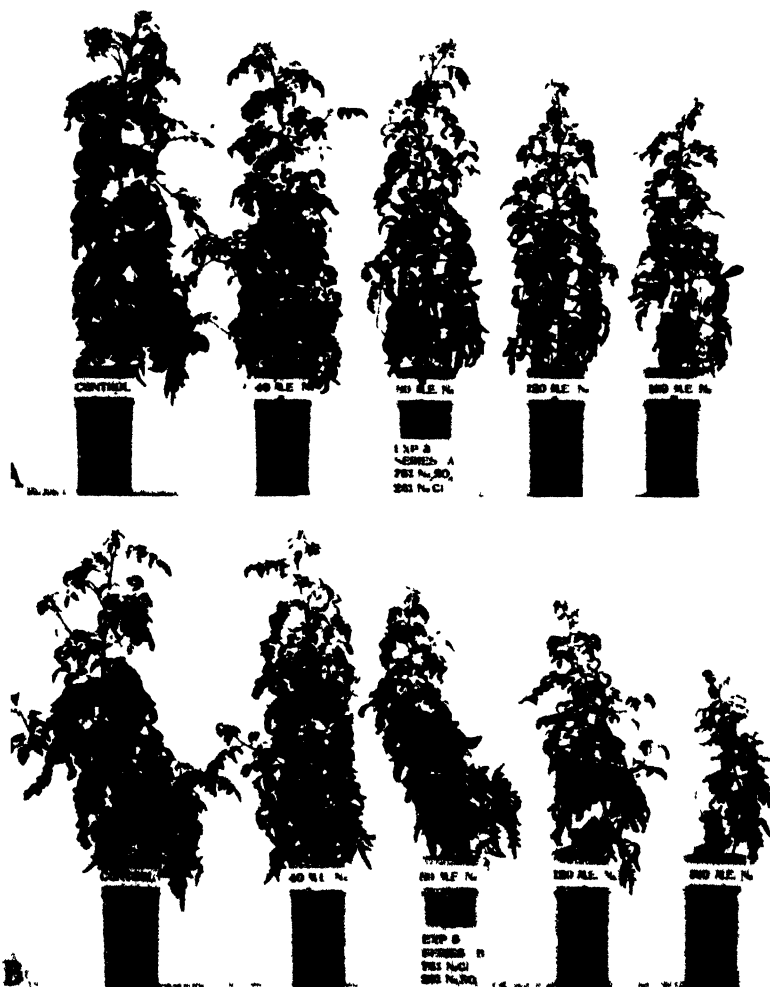
Fig. 1. Growth response to treatment with sodium salts. A. Series A (high sul-
phate treatments); B. Series B (high chloride treatments). The osmotic concentration
for each sodium treatment is shown in figure 6. Photographed five weeks before the final
harvest.

All cultures were started with the base nutrient solution; and, after the
seedlings were well established, sodium salts were added by 20-m.e. incre-
ments at two-day intervals to bring the respective treatments to full concen-
tration. Seven changes of the solutions were made during the course of the

experiment to maintain the osmotic concentration within a 0.5 atm. range
and to keep the nutrient ions above deficiency levels. The H-ion concentra-
tion was adjusted daily to a pH of 7.0 with HNO_3. The volume of the cul-
ture solutions was maintained by the addition of tap water daily, or twice
daily when transpiration was high.

PLANTING, THINNING, AND HARVEST DATES

The seeds were planted July 8, 1940, and six plants per culture were
maintained until August 6, when four were harvested. On August 28, one
of the plants was harvested, and the remaining one was carried to the final
harvest on November 8. To conserve space and permit a better analysis of
flowering and fruit development, the vines were pruned to single stems
(fig. 1).

TABLE II

FRESH AND DRY WEIGHTS, PERCENTAGE OF DRY MATTER, AND OSMOTIC CONCENTRATION OF
EXPRESSED JUICE OF VINES

SERIES AND CULTURE	TREAT- MENT	HEIGHT	HARVEST, 8–28–40		FINAL HARVEST, 11–8–40			
			FRESH WT.*	OSMOTIC CONC. JUICE	FRESH WT.*	DRY WT.	DRY MATTER	OSMOTIC CONC. JUICE
	m.c. Na/l.	cm.	gm.	atm.	gm.	gm.	%	atm.
A & B–1	Control	65	494	10.7	2155	353	16.4	11.6
A–2	40	67	343	11.2	1910	327	16.9	12.4
A–3	80	60	244	11.9	1664	285	17.1	13.6
A–4	120	51	151	12.7	1180	204	17.3	15.0
A–5	160	46	104	13.7	797	142	17.8	15.0
B–2	40	64	310	12.6	1832	324	17.7	13.7
B–3	80	57	206	12.9	1662	261	15.7	14.9
B–4	120	45	117	13.3	1091	181	16.6	16.4
B–5	160	35	74	15.3	443	75	17.0	15.9

* All weights are averages of tops per plant.

METHODS OF ANALYSIS OF JUICE

In order to determine to what extent the ions were accumulating, and to
get some indication of the effect of the salt treatments on carbohydrate syn-
thesis, analyses of the juice of the tops and of the fruit were made for the
principal ions, reducing sugar, and organic nitrogen. Samples of vegetative
material and fruit were rapidly frozen with dry ice and placed in cold
storage until analyses could be made. The material was then thawed, the
juice expressed with a Carver press under 2000 pounds pressure per square
inch, and the freezing point depression of the juice determined. The juice
was centrifuged for 15 minutes at 1800 r.p.m., hydrogen-ion concentrations
determined, and chemical analyses made by A.O.A.C. methods except for K^+
which was assayed by the HIBBARD and STOUT procedure (7). Analyses
were run for reducing sugars. Non-reducing sugars were not found in sig-
nificant quantities. The sap was cleared by the method described by HASSID

(5), and the sugar determinations were made by a modified HARDING and DOWNS procedure as outlined by VAN DER PLANK (12).

Results

VEGETATIVE RESPONSES AND GROWTH DATA

Growth data were obtained at the intermediate and final harvests. The higher the concentration of the culture solution, the greater was the reduc-
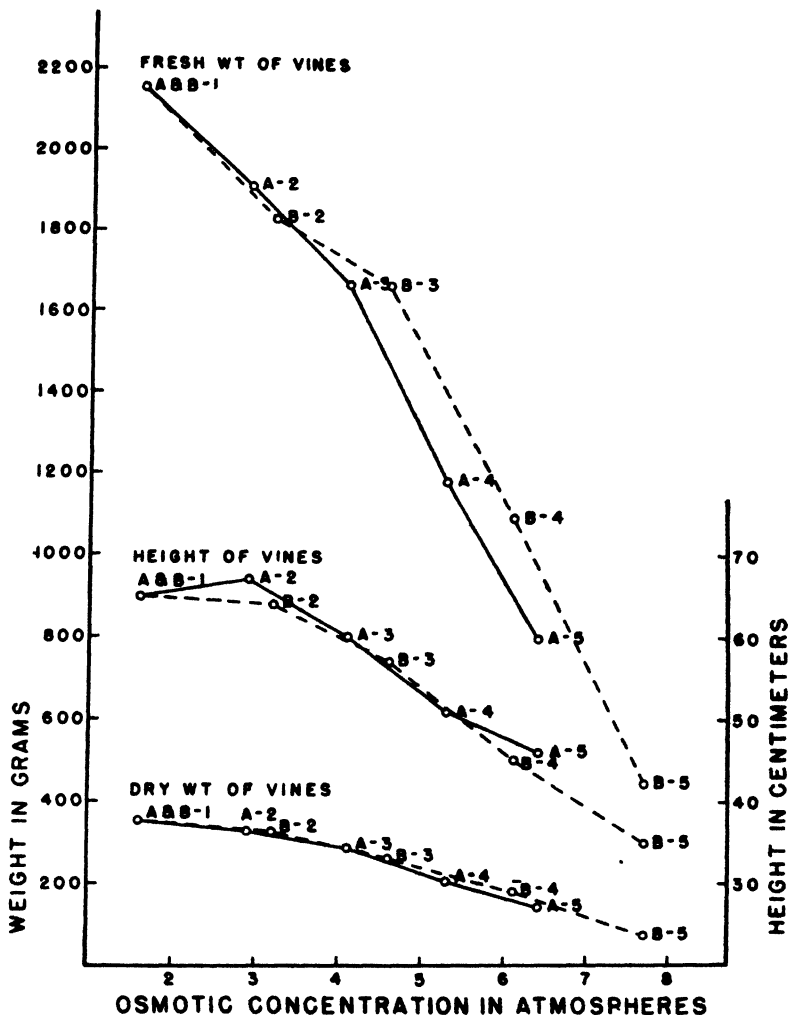
FIG. 2. Height, and fresh and dry weight of vines, plotted against osmotic concentration of the substrate. The letters A and B refer to salt treatments as shown in table I. Series A, high sulphate treatments; and series B, high chloride treatments.

tion in the height of stems and fresh and dry weight of vines (table II). At equivalent concentration of sodium, plants in the high chloride series showed greater growth inhibition than those in the high sulphate series (figs. 1

and 4). This might suggest that the toxic effect of the Cl^- ion exceeds that of the $SO_4^=$ ion, but it should be recognized that the activity of the Cl^- ion is much higher than that of the $SO_4^=$ ion in the respective solutions. Osmotic concentration is possibly the dominant factor in this difference in growth response since, at corresponding levels of sodium treatment, the osmotic concentration of the culture solution is greater in the chloride series, the
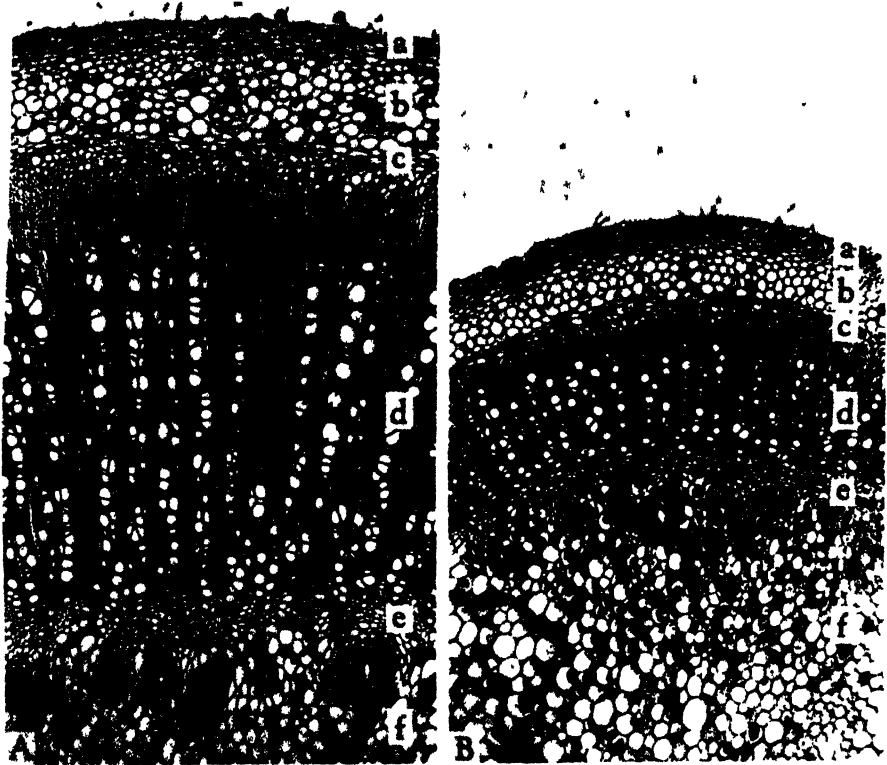
FIG. 3. Histological responses to high concentrations of sodium salts. Comparable sectors of transections of the middle internode of the tomato vine. A. Control culture (A-1) 1.6 atm. osmotic concentration; B. High salt treatment (A-5) 160 m.e. Na/l., 6.4 atm. osmotic concentration. The high salt stem has smaller, thicker-walled collenchyma (a), pericyclic fibers (c), and secondary xylem elements (d). The parenchymatous cells of the cortex (b) and pith (f) are smaller in the high salt stem, and those of the pith contain more starch. (The larger cells of the pith in the control stem are not shown in the figure.) The smaller size of the zone of secondary vascular tissues in B, from pericycle (c) to primary xylem (e), is the result of a slower rate of cambial activity at high osmotic concentrations, and the maturation of cells of smaller size. A and B at same magnification

differences ranging from 0.3 atm. with 40 m.e. of added salt to 1.3 atm. at the 160 m.e. level (table I). When height of stems and fresh and dry weight of vines are plotted against the osmotic concentration of the culture solutions, the differential effect of the Cl^- and $SO_4^=$ ions is very small (fig. 2). In fact, at isosmotic concentrations, there is little difference in the growth inhibition as expressed in terms of height of stems and dry weight of vines,

and the chloride plants (series B) had greater fresh weights of vines than did the sulphate plants (series A) at the higher levels. This is probably due to the tendency of plants to be more succulent when grown in high chloride than in high sulphate solutions.

ANATOMICAL RESPONSES

The anatomical and histological responses observed were similar to those ·)ted in an earlier study with high concentrations obtained by additions of !ium salts to a base nutrient solution and by multiplying the constituents
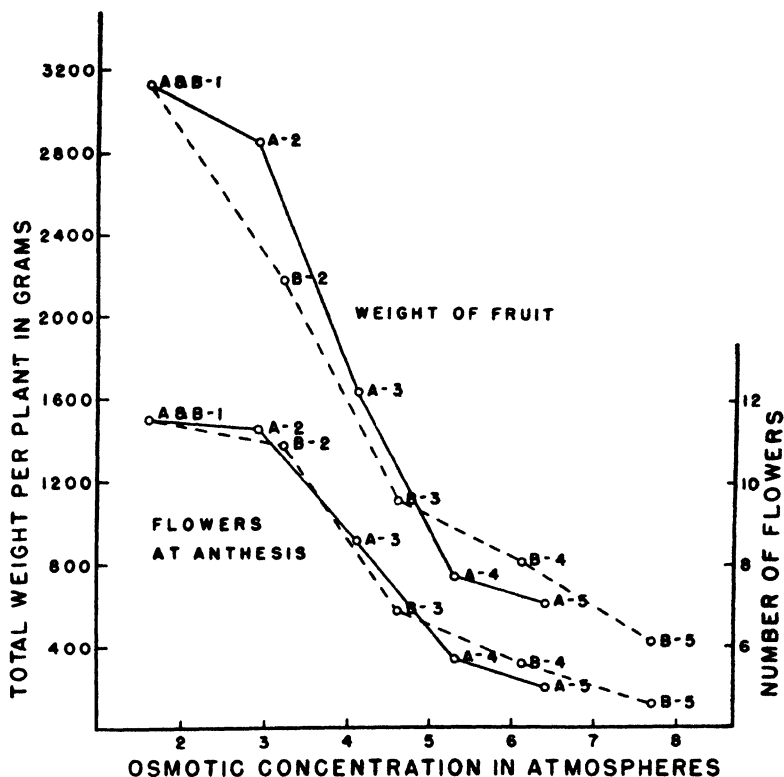
FIG. 4. Number of flowers at anthesis, daily average for 30 days, and total weight of fruit per plant plotted against osmotic concentration of the substate. The letters A and B refer to salt treatments as shown in table I. Series A, high sulphate treatments; and series B, high chloride treatments.

of the base nutrient (6). The smaller stems of vines grown at the high sodium levels as compared with the control vines were the result of decreased cambial activity, consequent reduction in the amount of secondary vascular tissue differentiated, and the smaller size of the mature cells. This was especially noticeable in the secondary xylem vessels and cells of the mechanical tissues of the high salt plants which were much smaller and had proportionately thicker walls. There was also a marked reduction in the amount of cortical and medullary parenchyma and in the size of the cells of those

TABLE III

FLOWERING AND FRUITING RESPONSES

| SERIES AND CULTURE | TREAT-MENT | FLOWERS AT ANTHESIS* | FRUIT SET† | FRUIT HARVESTED | | | |
				NO. PER PLANT	TOTAL WT. FRUIT PER PLANT	WT. PER FRUIT	BLOSSOM-END-ROT
	m.e. Na/l.				gm.	gm.	%
A & B–1	Control	11.5	188.5	56.5	3124	55.2	16.8
A–2	40	11.3	207	57.0	2856	50.1	33.3
A–3	80	8.6	136	55.7	1638	29.4	39.5
A–4	120	5.7	72	33.7	743	22.1	18.8
A–5	160	5.0	73	30.0	615	20.5	36.7
B–2	40	10.9	197	55.7	2183	39.2	29.9
B–3	80	6.9	111	39.3	1115	28.4	44.1
B–4	120	5.6	72	34.0	816	24.0	41.2
B–5	160	4.6	28	16.0	427	26.7	18.8

* Daily average, 30 days.
† Average per replication.

tissues. At the high salt concentrations, the accumulation of starch in the medullary and ray parenchyma was greater than in the control plants (fig. 3).

FLOWERING AND FRUITING RESPONSES

Evident flower buds were observed first in the control and low salt cultures and last on plants under high salt treatments. With one exception,
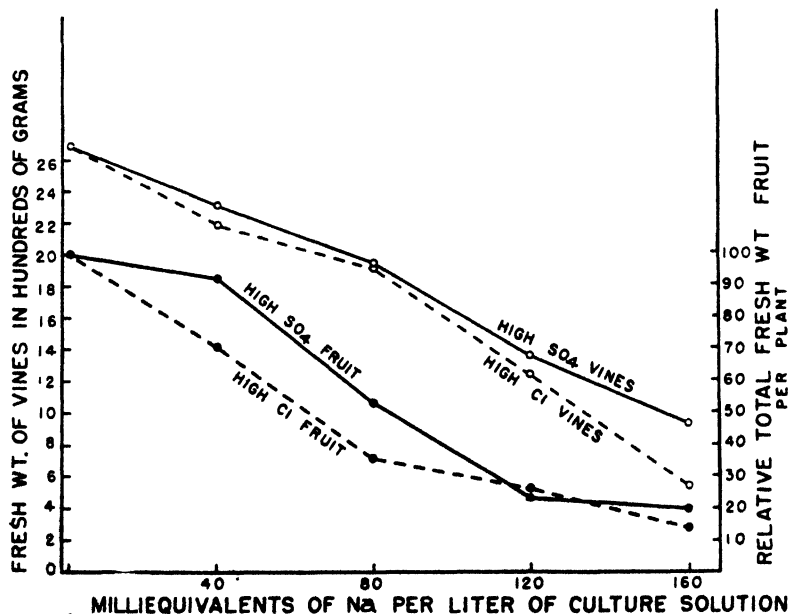
FIG. 5. The fresh weight of the vines and the relative total fresh weight of the fruit per plant for series A (high sulphate treatments) and series B (high chloride treatments).

this sequence also obtained for anthesis, the lag ranging from 3 to 6 days. From August 20 to September 24, floral counts were made to determine daily averages of flowers at anthesis per plant under each treatment. In both series, there was a marked decrease in the number of flowers formed with increasing concentrations of salt, but there was no significant difference in the effect of the Cl^- and SO_4^- ions (fig. 4). The total weight of fruit per plant and weight per fruit were reduced by the salt treatments. The reduction in total weight of fruit was not great at the 40 m.e. level in series A, but there was a sharp drop at higher salt concentrations (table III). The crop at the 120 m.e. level was about 25 per cent. of that of the control, and less than 15 per cent. at the 160 m.e. salt level in the B series (high chloride) (fig. 5).

On the basis of osmotic concentration, the high sulphate plants (series A) produced a greater total weight of fruit per plant than those of the high chloride series (B) at the lower osmotic values, but the reverse was true at the upper levels of salt concentration (fig. 4). Like the vegetative responses, the production of fruit indicates that the osmotic concentration of the substrate is more significant than the specific effect of the Cl^- and SO_4^- ions.

The incidence of blossom-end rot was high in all treatments. ROBBINS (11) found that approximately 80 per cent. of fruits on plants grown with solutions adjusted to 1.7 and 3.1 atm. osmotic concentration developed blossom-end rot and suggested that it was associated with wide fluctuations in the rates of evaporation, and occurred when the rate of transpiration was high. We observed that it was possible to reduce the amount of blossom-end rot by bringing the solutions up to volume frequently, thereby reducing the variations in water stress.

ANALYSIS OF JUICE OF VINES

The osmotic concentration of the juice of the vines increased as the vines matured. In every case, the juice of vines harvested in November was more concentrated than that for equivalent plants harvested in late August. At the final harvest, the osmotic concentration of the juice was greater at high concentrations of the culture solution, except at the highest levels where there was little change or a slight reduction as compared with the next lower level of salt treatment. Equivalent concentrations of sodium resulted in a higher osmotic concentration in the sap in the chloride than in the sulphate series, following the relationship of the culture solutions in this respect (fig. 6).

Calcium, magnesium, and potassium were supplied to all cultures in equal amounts, sodium being progressively increased from 1.6 m.e./l. (average content in Riverside tap water) to 160 m.e./l. In general, the total cations present in the juice tended to be uniform regardless of treatment. With one exception (B-5), there was an increase in sodium at each succeeding higher sodium treatment and this was accompanied by a decrease in the
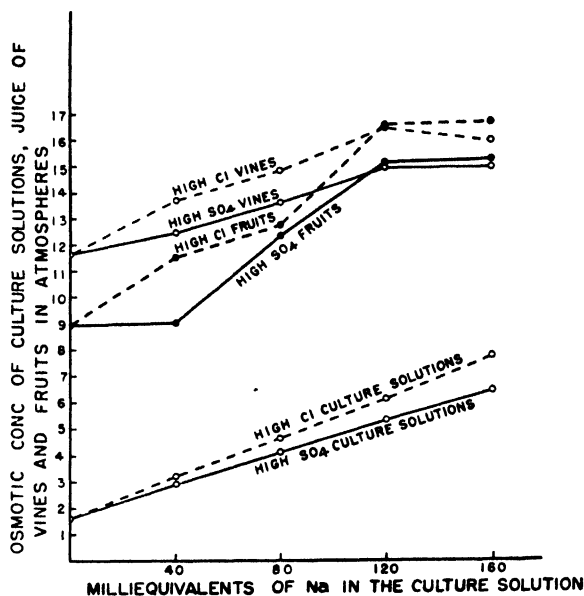
FIG. 6. The osmotic concentrations for the culture solutions, juice of vines, and juice of fruit at the different levels of sodium treatment. The high sulphate curves refer to series A, high chloride curves to series B.

concentration of other cations in the juice. In the A series (high sulphate) with increasing increments of sodium salts, the reduction in the amounts of Ca, Mg, and K was progressive. In the B series (high chloride), the relationship was not so clear owing to anomalous results from the B–3 cultures. With one exception (B–5), chloride and sulphate increased in the vines with increasing concentration in the culture solutions, the proportion of the two sodium salts in them being reflected in the greater SO_4 accumulation in the vines of the A series and the increased Cl concentration in those of the series B (table IV).

TABLE IV

ANALYSIS OF JUICE OF TOPS

SERIES AND CULTURE	TREAT-MENT	Ca	Mg	Na	K	SO₄	Cl	N*	REDUCING SUGAR
	m.e. Na/l.	m.c./l.	m.e./l.	m.c./l.	m.e./l.	m.e./l.	m.e./l.	m.c./l.	%
A & B-1	Control	81	156	10	163	113	13	92	1.42
A-2	40	97	140	55	135	136	41	83	1.24
A-3	80	69	139	110	115	164	65	96	1.20
A-4	120	51	130	135	117	171	92	117	1.17
A-5	160	48	111	151	112	174	92	130	1.21
B-2	40	85	158	75	120	118	102	104	1.47
B-3	80	50	115	121	101	108	162	95	1.00
B-4	120	70	127	150	110	122	184	112	1.17
B-5	160	70	128	114	112	120	168	111	1.11

* Organic and ammoniacal N.

TABLE V

ANALYSIS OF JUICE OF RIPE FRUIT. OSMOTIC CONCENTRATION, ACCUMULATION OF IONS, N, AND PERCENTAGE N, REDUCING SUGAR AND ACID

SERIES AND CULTURE	TREATMENT	OSMOTIC CONC.	Ca	Mg	Na	K	SO$_4$	Cl	N*	NITROGEN	REDUCING SUGAR	ACID†
	m.e. Na/l.	atm.	m.e./l.	m.e./l.	m.e./l.	m.e./l.	m.e./l.	m.e./l.	m.e./l.	%	%	%
A & B-1	Control	8.9	1.4	8.2	6.9	69.2	4.5	3.5	99	0.14	3.35	0.52
A-2	40	9.0	1.4	7.7	5.5	64.6	5.6	6.9	81	0.11	3.88	0.53
A-3	80	12.3	2.0	9.3	6.6	77.5	7.6	10.5	106	0.15	5.38	0.61
A-4	120	15.1	1.4	10.1	8.7	108.2	9.7	14.2	158	0.22	6.19	0.82
A-5	160	15.2	4.5	17.5	11.5	115.8	11.1	18.2	179	0.25	5.78	0.88
B-2	40	11.5	1.3	9.1	6.5	80.3	5.2	13.4	110	0.15	4.06	0.62
B-3	80	12.7	2.4	11.3	7.1	92.4	5.7	18.4	125	0.17	4.70	0.63
B-4	120	16.5	7.1	14.4	12.4	119.4	9.5	23.7	206	0.29	5.73	0.84
B-5	160	16.6	7.0	17.1	12.7	121.2	8.5	31.5	209	0.29	5.33	0.86

* Organic and ammoniacal N.
† Calculated as citric acid.

Organic nitrogen increased in plants grown under high sodium treatments, but there was little or no increase at intermediate levels. Reducing sugars were lower in the salt treatments than in the controls except in the B–2 culture, but the differences between treatments were slight (table IV)

ANALYSIS OF JUICE OF RIPE FRUIT

In ripe fruits, the osmotic concentration of the expressed juice incr̄ꜱ with increasing concentration of the sodium salts in the nutrient soli̇ ꞏ and, like the vines, the osmotic concentrations were higher in the B sꜱ̇ corresponding levels of treatment. As CHANDLER (1) and ROBBIꜱ̇ have pointed out, in most cases the osmotic concentration of the jꞏ ꞏꞏ vegetative parts tends to exceed that of the fruit. At control, loꞏ ꞏ intermediate levels of sodium concentration where the osmotic concentꞏ of the substrate ranged from 1.6 to 4.6 atm. we found that the osmotꞏ̇ centration of the vegetative sap exceeded that of the fruit by one to tꞏ atm. At the higher levels of salt treatment, however, in which the osmoꞏ concentrations of the culture solutions were from 5.3 to 7.7 atm., the osmoꞏ concentration of the fruit juice equaled or exceeded that of the vegetativé sap (fig. 6).

These relationships suggest that hydrostatic stresses may be operating to limit hydration which, as MACDOUGAL (10) has pointed out, is fundamental to growth. It seems probable that the growth of fruit at the highest salt levels was inhibited not so much by lack of food reserves as by inadequate hydration. As indicated in table V, the organic nitrogen in the juice of the fruit increased progressively up to the highest level of salt treatment; and the percentage of reducing sugar was highest under the 120 m.e. Na/l. treatment and only slightly less than that peak under the highest salt concentrations.

As compared with the vegetative tissues, the accumulation of Ca, Mg, Na, SO_4, and Cl in the fruit was low, and differences as the result of treatment were small (table V). Potassium and nitrogen occurred in larger amounts, the former approximating the accumulation in the vegetative saps at the high salt levels. With one exception, nitrogen values of the fruit juices were progressively higher with increasing concentration. The accumulation of sodium in the juice of the fruit increased with increasing increments of sodium in the substrate except for a slight decrease in the A–2 culture. The accumulations of SO_4 and Cl reflected the treatments, more of the former occurring in the A series, and of the latter in the B; but the amounts present were small as compared with the vegetative juice and with the concentration of these ions in the substrates in which the plants were grown (table V).

Discussion

Analyses of the fruit juices indicated that on a percentage basis, there was a slight increase in nitrogen, reducing sugars and mineral constituents with increasing concentrations of salts. Flavor and quality may be ad-

versely affected by the presence of Cl, SO$_4$, and Na ions and by the higher acid values that were found under high salt treatments, but the amounts of these ions accumulated as compared with their concentration in the vegetative sap were small. It should be added that no determinations were made for vitamin content of the fruit, such as those of HAMNER, LYON and HAMNER for ascorbic acid, so that we have no information as to how high salt tment may affect that aspect of the nutritional picture.

The principal deleterious effects of high salt concentration under the tions of this study appear to be marked reduction in the number of set and the weight and size of the fruits that reach maturity. Studies several varieties of tomatoes yielding results in line with those given have been reported by other investigators. ROBBINS (11) experimentvith the same variety used in our work, found that fruits produced on ts grown in nutrient solutions of 3.1 atm. osmotic concentration were .uch smaller than those grown at lower concentrations. LYON (8) using added Na$_2$SO$_4$ at 80 and 120 m.e./l. levels, found significant reductions in the mean weight of tomatoes of the Johannisfeuer variety and a reduction of 40 per cent. in the fresh weight of fruit produced at the higher salt level. EATON (3) growing the Stone variety at two concentrations of chloride and sulphate salts (50 and 150 m.e. l.) obtained 19 per cent. and 96 per cent. reductions in the relative dry weights of fruits at the low and high chloride levels respectively, and a 28 per cent. and 73 per cent. reduction with the corresponding sulphate treatments.

There also appears to be an increase in the incidence of blossom-end rot when the osmotic concentration of the substrate is high, although this condition is somewhat alleviated when fluctuations in water stress are reduced by keeping the concentration of the solution constant by frequent additions of water. Although our experience, like that of ROBBINS (11), indicates that irregularities in hydrostatic stress and the restriction of rate of absorption of water are probably the most important factors in the development of blossom-end rot, there is some evidence that the lack of accumulation, or the accumulation in excess, of certain ions may be contributing causes of this disorder.

On the basis of work reported here and additional unpublished data, it appears that the accumulation of potassium may be significant in blossom-end rot and that calcium may have an ameliorating effect. LYON, BEESON and BARRENTINE (9) using the Bonny Best variety and working with nutrient solutions of relatively low concentration found that "fruits produced in treatments where rot was most severe were low in calcium content and high in potassium and magnesium content." Our data are not directly comparable to those of the workers just mentioned since we were concerned with very high concentrations of total ions and of K, Mg, and Ca, and they were working at much lower levels. EATON (3) using Stone tomatoes in sand culture has obtained data that suggest that "calcium and magnesium accumulations singly or combined were important contributing factors" to the incidence of blossom-end rot.

Summary

1. Marglobe tomatoes were grown in sand cultures using a nutrient solution with NaCl and Na_2SO_4 as the added salts. Two series were set up with five treatments each: control, 40, 80, 120, and 160 m.e. Na/l. To segregate supplementary effects of the anions, series A had the sodium supplied as 25 per cent. Cl⁻ and 75 per cent. $SO_4^=$, series B as 75 per cent. Cl⁻ and 25 per cent. $SO_4^=$. The plants were grown to maturity to obtain data on floweri·· and fruit production.

2. The osmotic concentration of the substrate appears to be a primary factor in growth inhibition, although secondary effects of the Cl⁻ and S⌒· are noted.

3. The principal effects of high concentration of the substrate were:
   a. Reduction in height and diameter of stems and fresh and dry weight of vines.
   b. Reduction in cambial activity, maturation of cells of smaller size and relatively thicker walls in xylem elements and mechanical cells.
   c. Inhibited floral development and reduced set of fruit.
   d. Reduction in total yield of fruit and in size and weight per fruit.
   e. Increase in osmotic concentration of the vegetative and fruit juices.

4. The accumulation of Ca, K, Mg, and N in the juice of the ripe fruit was greater at high osmotic concentrations of the substrate although those elements were supplied in equal amounts for all treatments.

5. The accumulation of Cl, SO_4, and Na in the vegetative and fruit juices increased with increasing increments of salt in the substrate.

6. Flavor did not appear to be impaired by salt treatments.

7. The incidence of blossom-end rot appeared to be related to wide fluctuations in water stress. It is suggested that high accumulation of potassium may be a contributing factor.

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LITERATURE CITED

1. CHANDLER, W. H. Sap studies with horticultural plants. Missouri Agr. Exp. Sta. Res. Bull. 14. 1914.
2. EATON, F. M. Plant culture equipment. Plant Physiol. **16**: 385–392. 1941.
3. —————. Toxicity and accumulation of chloride and sulphate salts in plants. Jour. Agr. Res. **64**: 357–399. 1942.
4. HAMNER, K. C., LYON, C. B., and HAMNER, C. L. Effect of mineral nutrition on the ascorbic-acid content of the tomato. Bot. Gaz. **103**: 586–616. 1942.
5. HASSID, W. Z. Determination of reducing sugars and sucrose in plant materials. Ind. & Eng. Chem. Anal. Ed. **8**: 138–140. 1936.

6. HAYWARD, H. E., and LONG, E. M.   Anatomical and physiological responses of the tomato to varying concentrations of sodium chloride, sodium sulphate, and nutrient solutions.   Bot. Gaz. **102**: 437–462.   1941.

7. HIBBARD, P. L., and STOUT, P. R.   Estimation of potassium by titration of the cobaltinitrite with potassium permanganate.   Jour. Assoc. Offic. Agr. Chem. **16**: 137–140.   1933.

LYON, C. B.   Responses of two species of tomatoes and the F_1 generation to sodium sulphate in the nutrient medium.   Bot. Gaz. **103**: 107–122.   1941.

——————————, BEESON, K. C., and BARRENTINE, M.   Macro-element nutrition of the tomato plant as correlated with fruitfulness and occurrence of blossom-end rot.   Bot. Gaz. **103**: 651–667.   1942.

MacDOUGAL, D. T.   Hydration and growth.   Carnegie Inst. Washington, D. C.   1920.

ROBBINS, W. REI.   Relation of nutrient salt concentration to growth of the tomato and to the incidence of blossom-end rot of the fruit. Plant Physiol. **12**: 21–50.   1937.

12. VAN DER PLANK, J. E.   The estimation of sugars in the leaf of the mangold (*Beta vulgaris*).   I. Methods for the estimation of fructose, glucose and sucrose.   Biochem. Jour. **30**: 457–462.   1936.

STIMULATION OF THE ONION ROOT BY ALTERNATING CURRENT

L. JOE BERRY AND ROSALIE C. HOYT

(WITH NINE FIGURES)

Introduction

Positive direct current sent up an onion root may polarize or stimula₁
the root, *i.e.*, increase or decrease, respectively, the positivity of the electric
potential of the distal contact measured with respect to the earthed proxi₁ ₁ ₁
contact. Current sent down the root may depolarize or less con ₁ ₁
stimulate the root, *i.e.*, decrease or increase the positivity of this elec₁
potential. The type of change which the current produces is dependen
at least in part, on the strength and duration of the current; the tempera-
ture and oxygen tension of the environment; the previous treatment; the
individual characteristics; and the segment of the root under investigation
(2, 3). Since the flow of direct current in a given direction may result in
oppositely oriented potential changes which appear to summate in certain
cases, it was believed that the use of alternating current would simplify the
observations by eliminating one of the two effects, *i.e.*, the polar effect. With
this in mind the present investigation was undertaken and the results indi-
cate that the simplification occurred only in part.

CLARK (5) found that alternating current (6v) applied to Avena coleop-
tiles diminished the PD of 25 sections mounted in parallel between two
agar blocks to which electrical connection was made. The direction of the
change in potential is in agreement with the results reported below but no
analysis of the action was attempted. AMLONG and BÜNNING (1) and
BÜNNING (4) found the threshold value for AC stimulation which resulted
in a decrease in the potential difference of roots and stems of several plants.
METZNER (6) claims to have demonstrated rectification in 50 cycle AC in
several tissues, but experimental details are lacking. This report indicates
that stimulation with DC or AC elicits apparently identical electrical
changes in the root, confirming many of the experimental observations pre-
viously described (3). Moreover, the environmental factors, oxygen and
temperature, as well as the previous treatment of the root, are found to
influence irritability.

Method

The apparatus described by BERRY and HOYT (3) was used for this inves-
tigation. The onion roots were grown in the usual manner and the experi-
mental procedures were unchanged except for the use of alternating current.
In experiments where both alternating and direct currents were applied at
intervals to the root the DC circuit previously diagrammed (2) was em-
ployed. The 60-cycle, 110-volt house current was led from a potentiometer
and connected to the root through a rotary switch. In series with the root

was a Leeds and Northrup type 2450 astatic dynamometer with telescope and scale. The sensitivity was 0.798 microampere (root mean square) per millimeter on the scale. The 60-cycle voltage applied across the root was a pure sine wave, as tested with a cathode ray oscillograph. The current through the root was also found to be a pure sine wave, with no apparent rectification. Reversal of the AC leads did not affect the results. An RCA Ultrasensitive DC Meter was used for measuring potentials. This meter could be read to ̊ ̊ millivolts (one-fourth scale division) and had a period of 3 seconds. As ̊ the previous papers (2, 3), potential differences were not determined during the application of current but were recorded at 30-second intervals at other times during any experiment. In preparing the curves for publinot all points were included if their omission produced no alteration the curves.

Results

EFFECT OF ALTERNATING CURRENT OF INCREASING STRENGTH ON THE POTENTIAL OF VARIOUS SEGMENTS OF THE ROOT

Alternating current of increasing strength was applied for thirty seconds every eight minutes to the first five millimeters of the root tip. The contacts were then raised successively to 3 and 8, 5 and 10, 10 and 15, and back to 0 and 5 millimeters above the tip of the same root. Insets W, X, Y, and Z in figure 1 show the positions of the contacts around the root. Typical results for this procedure are given respectively in curves A, B, C, D, and A′. The first 5 millimeters, curves A and A′, show a decrease in potential following the application of 3.2 microamperes. The magnitude of the response (decrease in potential) increased as the current was increased up to 7.2 microamperes, curve A. A similar response to this current after the other segments of the root had been tested is found in curve A′. With still greater current, 12.8 microamperes, the response was slightly less. This effect is described in more detail in a section below.

In the more basal segments of the root, stimulation occurred only with stronger current; 19.6 microamperes were required in curve B, 12.0 microamperes in curve C and 27.2 microamperes did not stimulate the most basal segment as shown in curve D. These results are in agreement with those reported for direct current (3) in which polarizations and depolarizations, rather than stimulations, were usually observed in basal segments. It should be stated, however, that in some roots a small response may sometimes be obtained even in basal segments but always the required current is higher and the magnitude of potential change is smaller than in the apical segment (fig. 8, curve B).

In one root it was found that the potential following the flow of alternating current increased (more positive) rather than decreased. The response of this root is shown in figure 2. Current was applied for 30 seconds. The initial potential of the apical 5 millimeters was about 10 millivolts *nega-* *tive* (curve A) and thus was oriented opposite to that of the majority of

roots. For this reason one might expect the reversed direction of the response but at the end of the series when the contacts were returned to the apical 5 millimeters (curve A') the polarity potential was between 30 and 40 millivolts *positive* and the response continued to cause an increase in potential. In most experiments in which a reversal of polarity of the apex occurred (the potential became negative), the current flow still produced ⌐ decrease in positivity (fig. 4, curve B between 100 and 120 minutes).
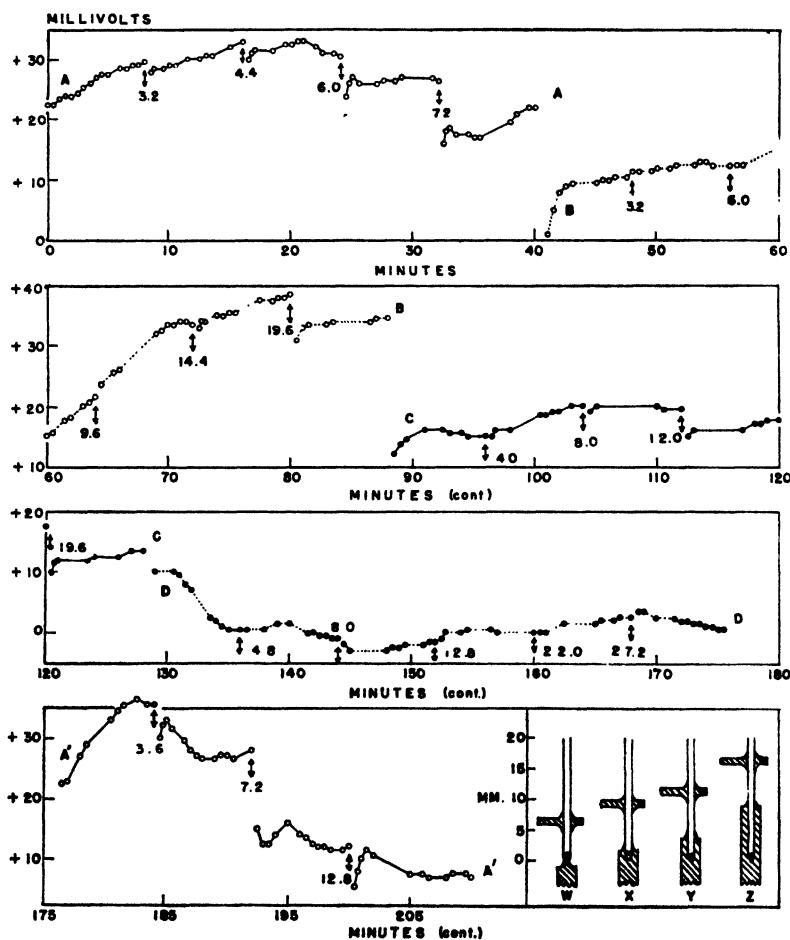
FIG. 1. The response of inherent potential to alternating current. Double-headed arrows indicate AC sent through the root for 30 seconds. The number with each arrow gives the magnitude of the current in r.m.s. (root mean square) microamperes. The positions of the contacts were: Curve A, 0 and 5 mm. (inset W); curve B, 3 and 8 mm. (inset X); curve C, 5 and 10 mm. (inset Y); curve D, 10 and 15 mm. (inset Z); curve A', 0 and 5 mm. All curves are from the same root.

Both curve B and curve C of figure 2, obtained with the contacts at 3 and 8, and 5 and 10 millimeters above the tip, respectively, insets Y and Z, gave the same type of response as the tip. The strength of current necessary was higher in each case than that required for curve A but the magni-

tude of change was approximately the same.   The abnormal behavior of this root must indicate an exceptional condition in the cells since it was the only one of dozens used during the course of the investigation that gave this interesting response.   It is hoped that further experiments will yield an explanation of the results.

Since figures 1 and 2 show the first five millimeters of the root tip to be most easily stimulated, it was considered desirable to determine which millimeter segment of the five was primarily responsible for the observed potential change.   The current was increased until an appreciable response was obtained and approximately this current was then applied in the same way
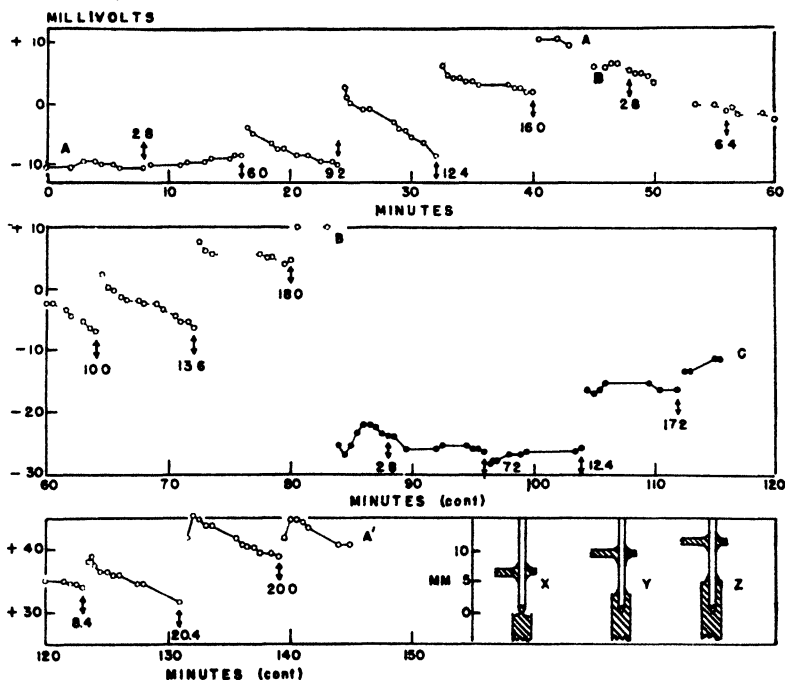
Fig. 2. Same as figure 1. The positions of the contacts were: Curve A, 0.0 and 5 mm. (inset X); curve B, 3 and 8 mm. (inset Y); curve C, 5 and 10 mm. (inset Z); curve A', 0 and 5 mm. All curves are from the same root.

twice each to the five one-millimeter segments in the first 5 millimeters of the root tip.   The contacts were then returned at the end to 0 and 5 millimeters above the tip and the same current applied twice and finally to 1 and 5 millimeters above the tip and current again applied.   Figure 3 gives the result typical of the four roots tried.

Curve A is for the entire 5 millimeters and 10.4 microamperes produced a response of 11.5 millivolts.   With the contacts at 0 and 1 mm. above the tip, 11.2 microamperes gave a response of 14.5 and 19.5 millivolts (curve B). The slight increase in current resulted from the decreased impedance of the shorter segment of root and the inability to set the potentiometer so as to give a predetermined current.   Curve C had the contacts at 1 and 2 mm.

above the tip and 12.0 microamperes did not stimulate the segment. Similarly, curves D, E, and F with the contacts at 2 and 3, 3 and 4, and 4 and 5 mm. above the tip, respectively, gave no response with about 11.5 microamperes. When the contacts were returned to 0 and 5 mm., 10.8 microamperes produced responses of 24.5 and 16.0 millivolts. Finally, with the contacts at 1 and 5 millimeters above the tip no stimulation resulted from
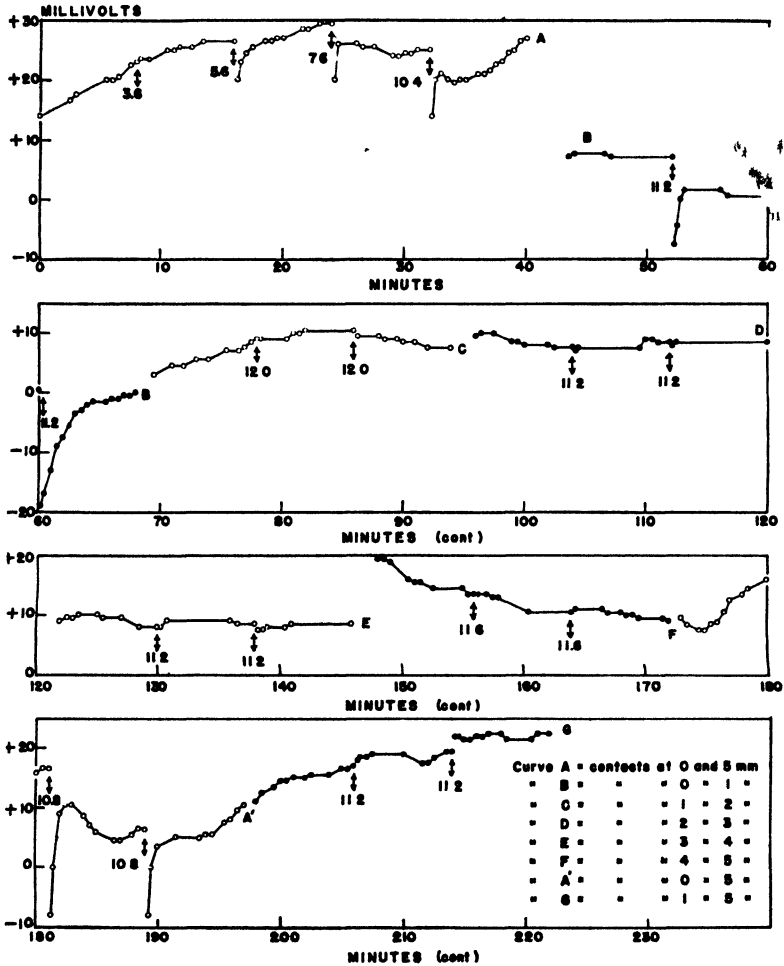
FIG. 3. The effect of segment length and position on the response to alternating current. Double-headed arrows indicate the AC sent through the root for 15 seconds. The number with each arrow gives the magnitude of the current in r.m.s. microamperes. The positions of the contacts are given in the table. All curves are from the same root.

current flow. Hence it is apparent that the first millimeter of the root tip is the part most easily stimulated with alternating current. With that segment removed from the circuit by raising the lower contact, no potential change resulted from the flow of what had been a stimulating current. In this case the results may be due to the greater current density over the first

millimeter of the tip but for more basal segments (fig. 1) the observed differences in response to current flow are not so simply explained.

Thus the flow of AC through a root normally decreases the positivity of the electric potential as measured but in exceptional cases the response to current flow may be oriented in the opposite direction. A response is more readily obtained in apical than in basal segments of the root and with smaller currents.

CT OF ALTERNATING CURRENT OF CONSTANT MAGNITUDE APPLIED
TO THE ROOT FOR INCREASING LENGTHS OF TIME

ing with a time of 5 seconds, an alternating current which proesponse was allowed to flow through the root and its effect on poten-érved. Eight minutes later the same current was passed through ιt for 10 seconds and so on with 5-second increments up to 30 seconds ιnally 60 seconds. Curves A and B, figure 4, are for the first 5 mm. ωo different root tips and curve C is for 5 and 10 mm. above the tip of ιe same root as that of curve A. In the latter, 9.6 microamperes for 5 seconds decreased the potential 41.5 millivolts and as the time of current flow progressively increased to 10, 15, 20, and 25 seconds the responses became smaller until finally neither the 30- nor the 60-second flow produced a change. A return to 5 seconds gave a decrease of only 9.5 millivolts (compared to the original 41.5 mv.), followed by no effect for 30 seconds and only 6.0 millivolts for another 5-second application. This change in the response of the root to current flow as the duration of flow increased took place with the magnitude of the potential essentially constant at about 20 millivolts positive after the first application. A similar effect was obtained with the segment of root 5 and 10 millimeters above the tip, curve C, but in this case no response resulted after a 25-second flow of 21.2 microamperes and only a 1.5 millivolt decrease was produced by the final 5-second application as compared to the initial 6.5 mv. change.

A slightly different effect is shown in curve B beginning with an application of 6.0 microamperes for 5 seconds that gave a 30 mv. response which gradually decreased to 7.5 mv. at both 20 and 25 seconds, increased to 15.5 mv. for 30 seconds, and to 12.0 mv. for 60 seconds. Again the return to a 5-second flow gave a smaller response (15.5 mv.) than originally as did the 30-second (5.0 mv.), and final 5-second flow (10.0 mv.). With the same series of current applications repeated using a larger current (10.8 microamperes) it is seen that the magnitude of response regularly decreased up to the 60-second flow, except for the 5.0 mv. greater decrease at 10 seconds than at 5 seconds. Here also the final 5-second flow produced as great a response as the initial one. The decrease in response that generally occurs as the duration of current flow increases is in close agreement with the similar experiments for direct current (3) save that in the latter case the potential change due to polarization is summed with the progressively smaller potential change due to stimulation and the net result may be an increase in potential when current passes up the root for longer time intervals.

The application of current of constant magnitude and duration does not produce a progressively smaller response if the period between applications permits recovery of the potential to approximately the original value (fig.
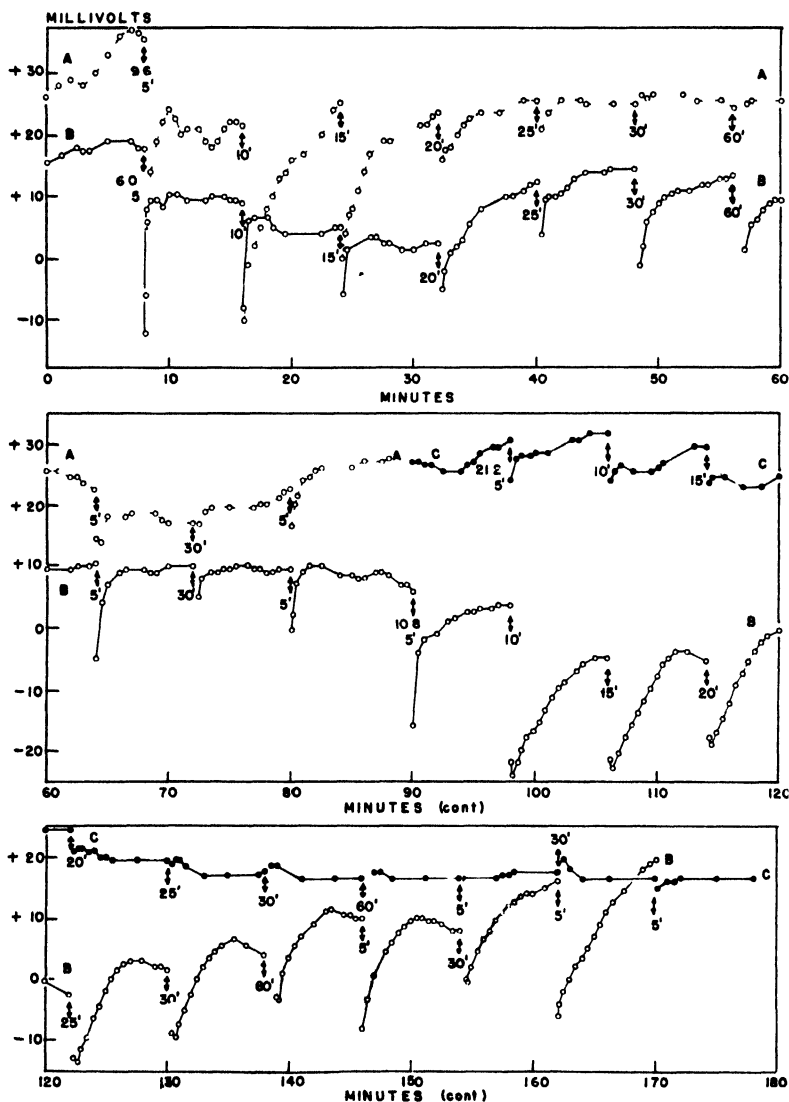
FIG. 4. The response of inherent potential to alternating current of constant magnitude but varying duration. Double-headed arrows indicate the application of AC to the root. The numbers with each arrow give the magnitude of the current in r.m.s. micro amperes, and the duration of current flow in seconds ('). When no magnitude is given it was the same as that last indicated. The curves A and C refer to one root, and curve B to another. The positions of the contacts were: Curve A, 0 and 5 mm.; curve C, 5 and 10 mm.; curve B, 0 and 5 mm.

5, first part of curve A). Even so, under such conditions, variations in the magnitude of potential-decrease following current flow may occur. Such

variations, however, are never as orderly as the decrease obtained with longer intervals of application.

EFFECT OF ALTERNATING CURRENT OF CONSTANT MAGNITUDE APPLIED WITH SHORTER TIME INTERVALS BETWEEN APPLICATIONS

A stimulating current was applied for 30 seconds to the first 5 mm. of the tip every eight minutes for four times. It was then passed through the root four times for the same length of time but with four minutes between,
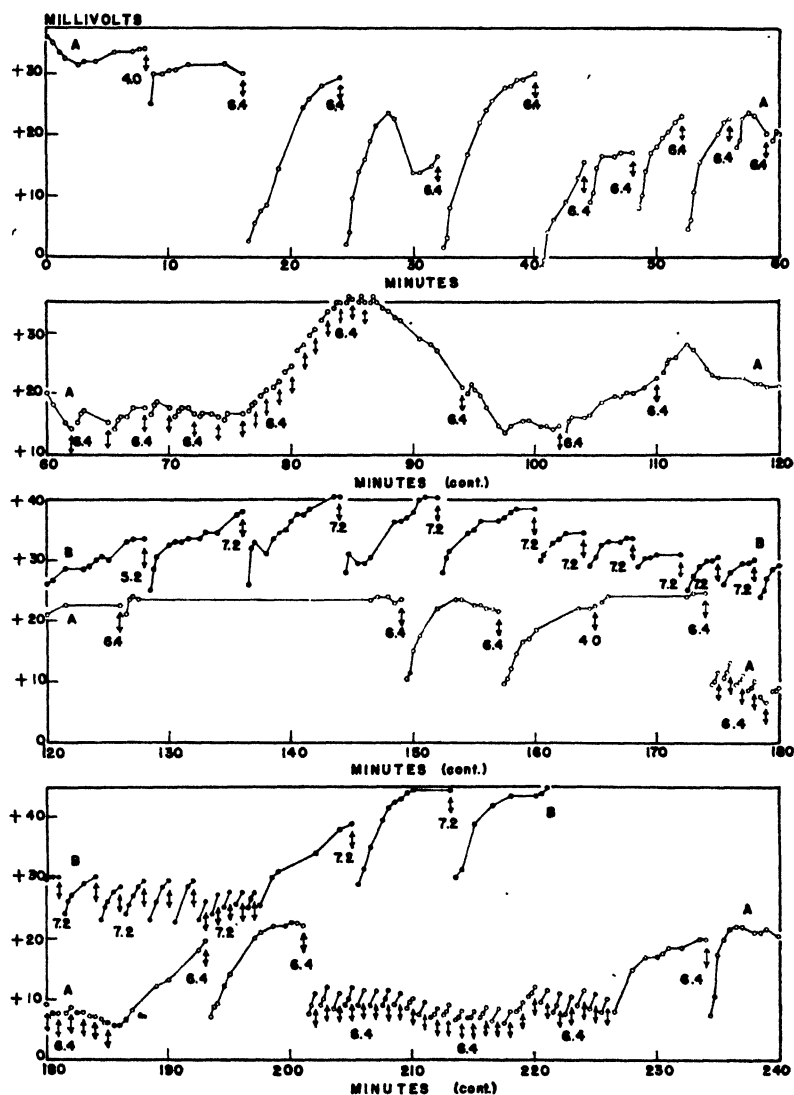
FIG. 5. The effect of decreasing the time between applications of alternating current. Double-headed arrows indicate the application of AC for 30 seconds. The numbers with each arrow give the magnitude of the applied current in r.m.s. microamperes. Curves A and B refer to two different roots. The positions of the contacts were: 0 and 5 mm. for both curves.

then similarly with three, two, and one minutes between applications. Of the four roots so treated two are shown in figure 5. The first part of curve A shows a behavior unlike the other three but the last part of that curve exhibits typical behavior. It should be noted that 6.4 microamperes gave a much larger decrease in potential than 4.0 microamperes and was therefore used for the series in curve A. The four applications of current with eight minutes between, result in three of four responses of about equal magnitude (30 mv.) with the third only half as great. The responses with four minutes between current flow increased in size the first three times and decreased the last. With three and then with two minutes between current applications, virtually no potential change took place. Because one minute intervals between current flow was accompanied by a potential increase, the series was extended until a constant potential was reached. This increase in inherent potential as a result of current flow and the failure of the root to respond at the three and two minute intervals is the exceptional behavior. Eight minutes following this last series the same current was again passed through the root and no response was produced. This "refractory state" persisted for 64 minutes as shown by an occasional application of current during this time and then at 150 minutes on the time scale, the root again responded. The magnitude of this and the following responses was approximately the same as the smallest of the initial four. The root even after the return of irritability was not as easily stimulated as originally since 4.0 microamperes failed to have any effect. Moreover, eleven applications of current one minute apart failed to produce the rise in inherent potential, and two applications eight minutes apart gave normal responses. This was finally followed by twenty-five applications of current at one-minute intervals and all but one caused a decrease in potential. The inherent potential returned to its initial level after this final series and eight minutes later the root was again stimulated by current flow. Thus after recovery of irritability a treatment more drastic than the one initially responsible for the "refractory" condition failed to cause another period of lost irritability.

The root in curve B of figure 5, which starts at 120 minutes on the time scale, required 7.2 microamperes for a good response. It was treated the same as the root in the first part of curve A and responded each time current passed through the root regardless of the length of the recovery period. At the end the original potential level was reached and the size of the response equalled the one at the beginning. The only noticeable effect of applying current with a short recovery period between applications in this case, as well as in the latter part of curve A, was to permit less recovery from the former stimulation and hence less potential decrease. Throughout the experiment in curve B the lower level of the potential immediately following current flow was remarkably constant at about 25 mv. positive. A similar condition is to be seen in curve A over the latter half where the potential was between 5 and 10 mv. positive.

Summarizing, it is found that AC applied several times in rapid succession prevents complete recovery and decreases the observed electric potential

to approximately the same initial level after each current flow. However, this treatment seems capable under some conditions of giving rise to a "refractory state" in the root during which time the applied current produces no response in the root.

EFFECT OF ALTERNATING CURRENT FOLLOWED BY DIRECT CURRENT ON THE POTENTIAL OF THE APICAL FIVE MILLIMETERS OF THE ROOT

BERRY and HOYT (3) have shown that a root tip stimulated by direct current could not be stimulated again when current of the same strength
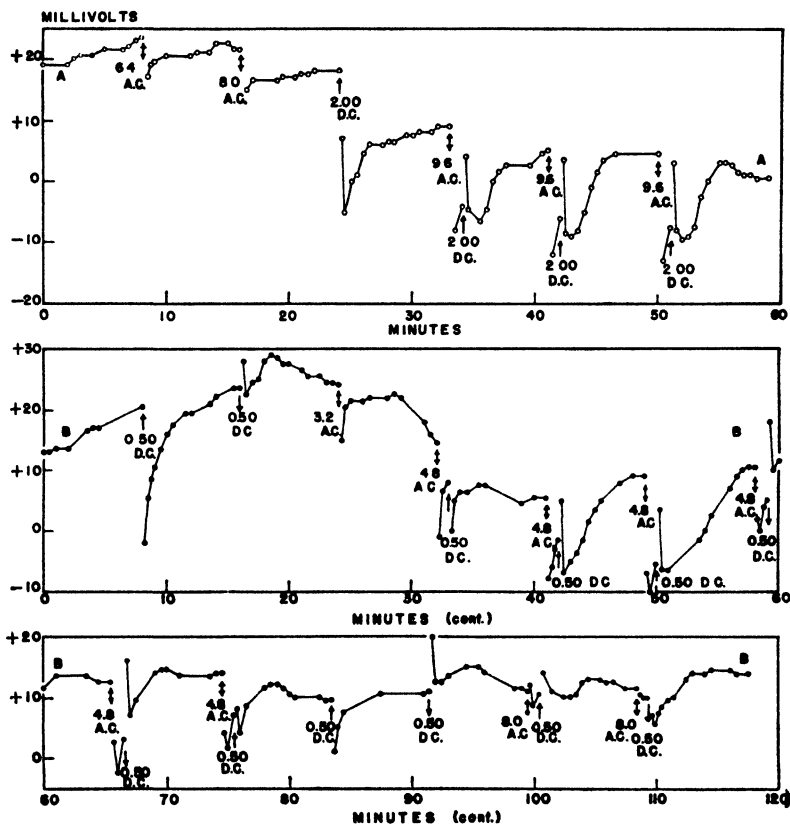
FIG. 6. The effect of alternating current on the response of inherent potential to applied direct current. Double headed arrows indicate the application of alternating current for 15 seconds, single-headed arrows indicate the application of direct current up or down the root for 15 seconds. The numbers at each arrow give the magnitude of the current (AC or DC) in microamperes. The positions of the contacts were 0 and 5 mm. for all curves. A different root was used for both curves.

was sent through the root within one or two minutes. Instead, the second application of current polarized the root when it opposed the inherent flow of current. In order to see whether a similar effect would result with AC, direct current of stimulating magnitude was sent up the root one minute after the potential was decreased with AC. Typical results with the con-

tacts 0 and 5 mm. above the tip are given from two different roots in figure 6. Current was applied for 15 seconds in all cases. The root that gave curve A could be decidedly stimulated with 2.00 microamperes DC and 9.6 microamperes AC. When the DC was applied one minute after the AC a polarization was produced which was almost perfectly repeated three successive times with eight minutes between the two current applications.

In curve B, 0.50 microampere DC stimulated the root when sent either up or down the root. The latter type of response (an increase in potential after direct current is sent down the root) is not as commonly seen as the former. This response of the root made it possible to determine the effect of both types of DC after AC stimulation. Direct current sent up the root one minute after 4.8 microamperes AC had stimulated it produced another decrease in potential. When repeated after an eight minute recovery period, the DC polarized the root. The same effect was observed after another eight minute period. When DC passed down the root after the AC application, an increase greater than that produced by DC alone was found. This was repeated with the same result. Another application of DC up the root, following the AC, at first increased the potential to a small extent which quickly fell below the initial potential. A repetition of the DC alone, once in each direction, showed the root still to be stimulated by each. Then by increasing the AC to 8.0 microamperes, practically no response was observed and the direct current application that followed increased the potential when sent up the root and decreased the potential when sent down the root.

Effect of alternating current of increasing magnitude on the irritability of the root to direct current

It has been suggested above (sections 2 and 3) that the root is capable of undergoing a decrease in irritability even to the extent of becoming "refractory" under some conditions of current application. This has been found to be the case when sufficiently strong AC is used on the first five millimeters of the root tip. Not only does the root become practically "refractory" to AC stimulation but also to DC stimulation. At the same time, the root can still be polarized with DC. Typical of the results from several different roots is that shown in figure 7. Current was applied for fifteen seconds each time and eight minutes elapsed between applications. Three different magnitudes of DC were first sent up the root and in each case a rather large decrease in potential was produced. Two applications of AC of increasing strength also stimulated the root and another DC (0.50 microampere) again decreased the potential but not as much as originally. By increasing the AC still more no change in potential occurred when 12.0 microamperes was used at 64 minutes on the time scale. This was followed by five applications of 0.50 microampere DC with the first two polarizing and the last three stimulating the root. When 12.0 microamperes AC was again sent through the root it produced a sizable response which became

successively smaller on the next two applications. Beginning at 136 minutes, the AC was increased each time it was applied and each response was smaller until no change resulted with both 40.0 and 47.2 microamperes. The
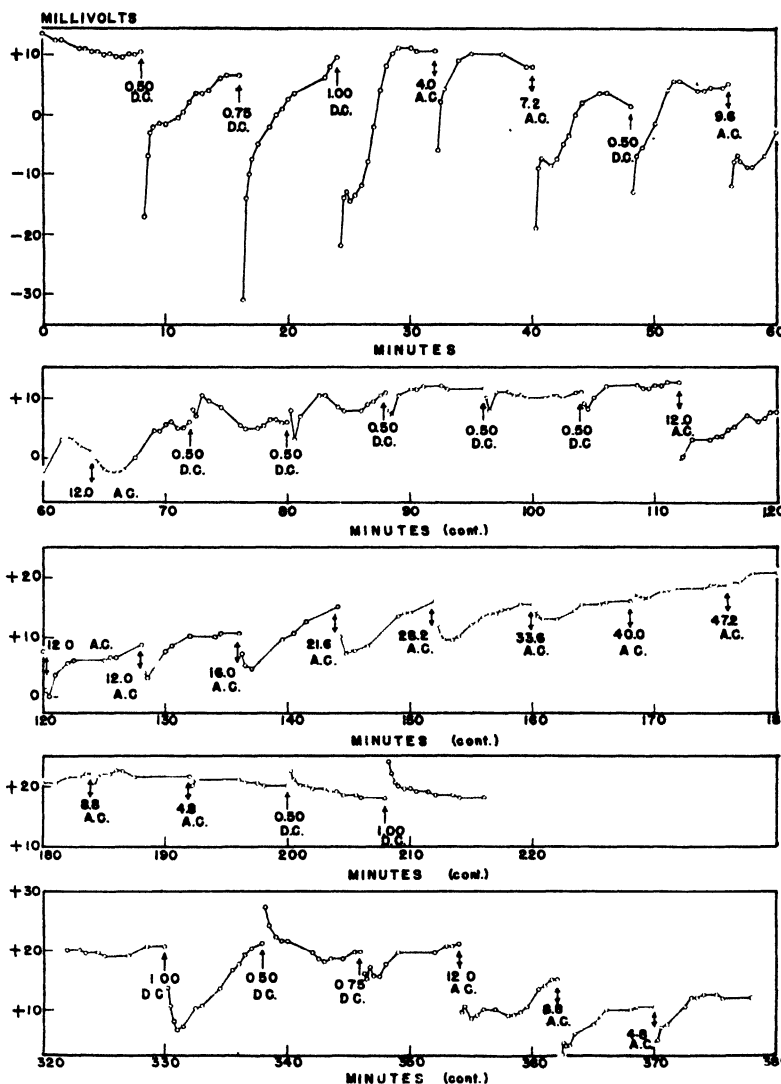
FIG. 7. The effect of alternating current of increasing magnitude on the response of inherent potential to applied direct current. Double-headed arrows indicate the application of alternating current for 15 seconds, single-headed arrows indicate the application of direct current up or down the root for 15 seconds. The numbers at each arrow give the magnitude of the current (AC or DC) in microamperes. The contacts were at 0 and 5 mm.

magnitude of AC which originally gave the largest responses now produced very small potential changes and both 0.50 and 1.00 microamperes DC polarized the root. *The root was now in a "refractory" state and this condition existed when the inherent potential was higher than at any previous period.*

In fact the increase in inherent potential took place during the hour (120 to 180 minutes) when the highest AC was being passed through the root. This increase in potential should be compared with the rise shown in curve A, figure 5, of this paper and that of figure 9 in the paper by BERRY and HOYT (3).

By permitting the root to remain undisturbed for a little over 100 minutes and then testing its response to current flow, it was found that a partial recovery of irritability had occurred. Both 1.00 and 0.75 microampere DC stimulated the root while 0.50 microampere only polarized it. With AC, 12.0, 8.8, and 4.8 microamperes all gave appreciable responses. This rather drastic treatment of roots frequently caused an angular bend in the first few millimeters of the tip by the following day but growth continued and no permanent damage beyond the bending seems to have occurred.

EFFECT OF TEMPERATURE ON THE RESPONSE OF THE ROOT TO ALTERNATING CURRENT

In order to compare the effect of temperature on AC stimulation with that for DC stimulation (3), an identical procedure was followed except for the difference in current used. Reference is made to that work for the technique used and a discussion of its limitations. Essential confirmation was obtained and two of the seven curves are presented in figure 8. Curve A is for the first five millimeters of the tip of one root and curve B is for the 10 to 15 millimeter segment of a different root tip. Current was applied for 30 seconds in all experiments. The first sizable response required 6.4 microamperes for curve A (small responses may be seen for both 3.2 and 4 8 microamperes) and 12.8 microamperes for curve B. After lowering the temperature to between 14° and 15° C. for one hour the apical segment showed a large response with 4.8 microamperes but no response for 3.2 microamperes. On the other hand, the basal segment was only stimulated to a lesser degree with nearly twice as much current as that originally required. As the temperature increased the magnitude of current necessary for an appreciable response also became greater in the apex and exceeded the original value. This condition might well have been due to the greater surface conductivity of the root resulting from condensation of water as the air in the chamber became warmer and more moist with the root temperature remaining lower. Finally, with the return to room temperature approximately the same magnitude of current stimulated the root as in the original condition. In the basal segment, however, the return to room temperature lowered the strength of the current required for stimulation to approximately its former value. However, with all roots the basal segment did not behave in such direct contrast to the apical segment and, as with DC stimulation, the effect of a lower temperature was much more pronounced and definite in the apex than in the base. .Thus as shown in curve A, the strength of current for stimulating an apical segment of root is decreased by lowering the temperature; this verifies the results obtained with DC stimulation.

EFFECT OF HYDROGEN AND OXYGEN ON THE RESPONSE OF INHERENT
POTENTIAL TO AC STIMULATION

When oxygen is removed from the environment of a root by passing
hydrogen through the chamber, direct current which stimulated the root
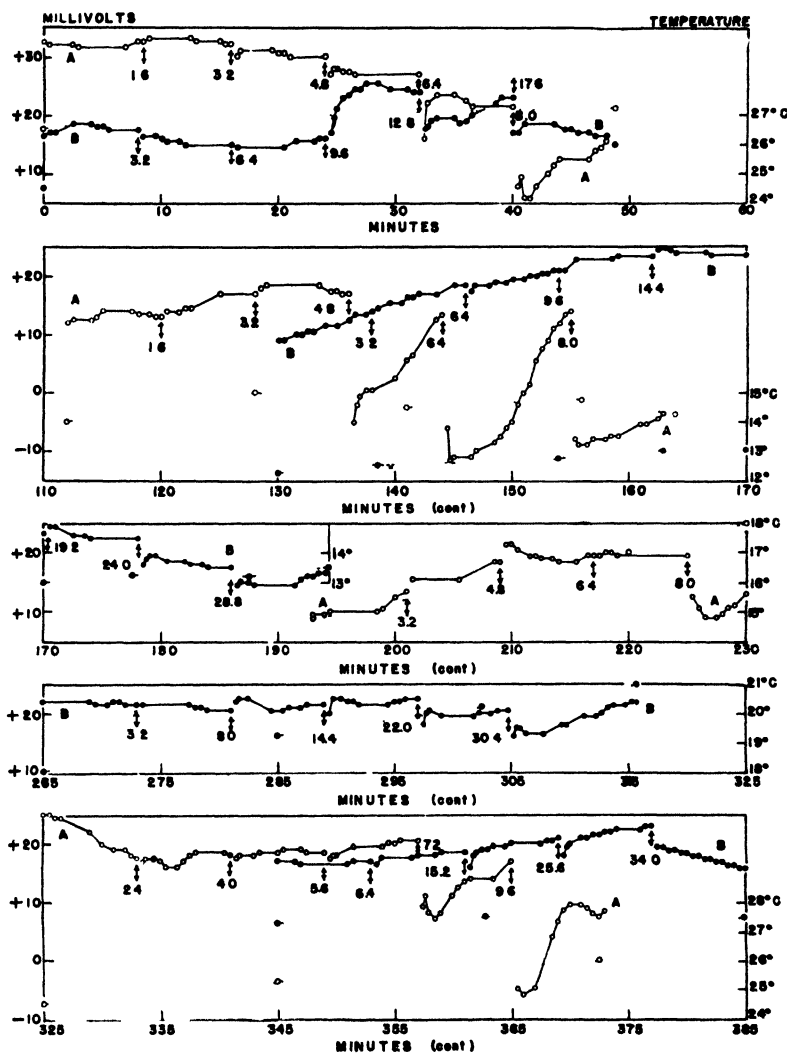
FIG. 8. The effect of temperature on the response of the root to alternating current.
Double headed arrows indicate the application of alternating current for 30 seconds.
The numbers beside each arrow give the magnitude of the current in r.m.s. microamperes.
The variation of temperature with time is given by the broken curves. Curve A and B
refer to two different roots, and the positions of the contacts were: curve A, 0 and 5
mm.; curve B, 10 and 15 mm.

only polarizes it. This effect is reversible by replacing the hydrogen with
oxygen (3). It was considered important to observe the change a similar
treatment would produce on the alternating current stimulation. The

curve in figure 9 is typical of this action and shows that without oxidative metabolism no stimulation was possible but that irritability returned with a return of oxygen. Moreover, the reversibility of the effect was shown by
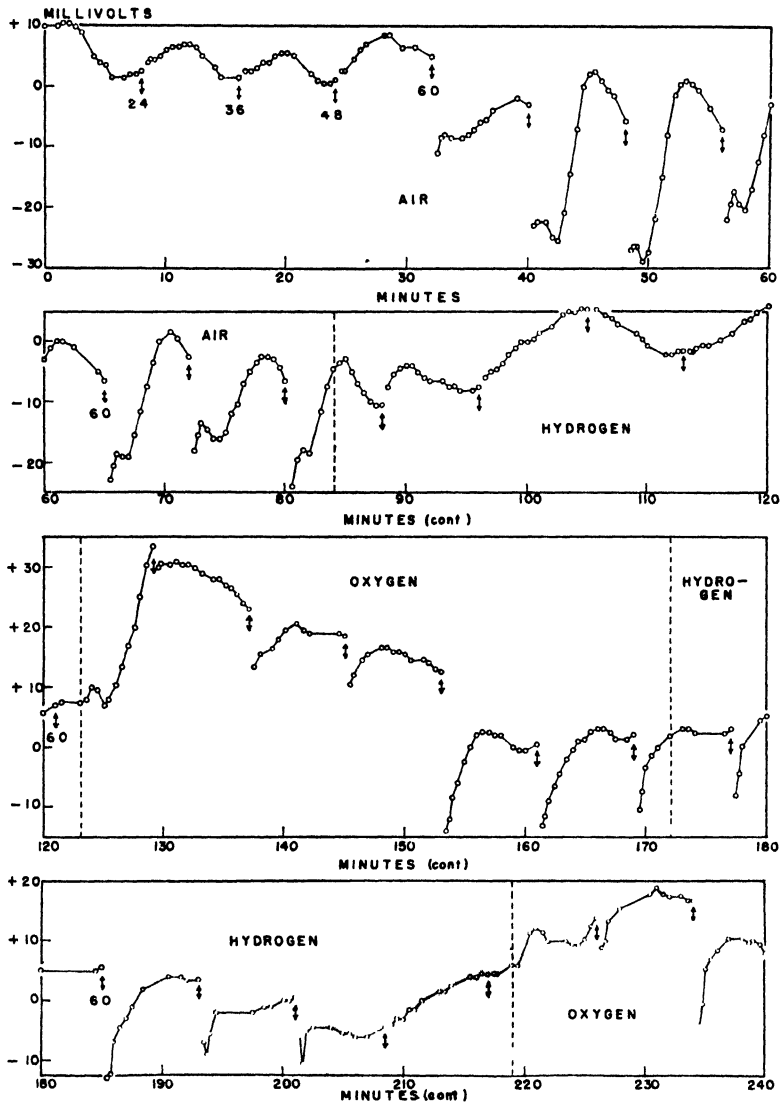
FIG. 9. The effect of hydrogen and oxygen on the response of inherent potential to alternating current. Double-headed arrows indicate the application of alternating current for 30 seconds. The numbers at the arrows give the magnitude of the current in r.m.s. microamperes (when no number is given the current was the same as that last indicated). The dotted vertical lines indicate the time at which the circulation of hydrogen or oxygen was begun. The contacts were at 0 and 5 mm.

repeating the oxygen removal and readmission. The second period in hydrogen required a longer time before the loss of irritability appeared; this might easily be attributed, however, to the fact that hydrogen had to replace

only air the first time and oxygen the second time. A sufficiently low oxygen tension would naturally be slower in appearing in the latter case. This particular curve is of additional interest because at the beginning of the removal of air by hydrogen at 84 minutes the potential was slightly negative and yet was responding to current flow with potential changes of approximately 20 mv. In hydrogen the potential at which no response occurred after applying AC was at first almost the same as before and later was even higher. Thus the absolute magnitude of potential is not directly involved as a cause and effect mechanism. This fact would not have been evident in the other roots of this series since normally there is a fall from a positive PD to essentially zero when hydrogen replaces air. The change in the response of a root to current flow, however, follows a similar pattern in all the roots tested.

Discussion

Alternating current when sent through an onion root could alter the observed electric potential: (1), as a result of rectification by the root comparable to the action of various tissues reported by METZNER (6); or (2), by stimulating the root, thereby producing a greater negativity in observed potential similar to the effect sometimes observed with direct current (3). Neither of these explanations can fully account for all of the experimental facts as shown by the analyses that follow.

The rectifying element in the root would have to possess the following characteristics: (a) its resistance to upward flow of current should be greater than its resistance to downward flow of current, thereby normally resulting in depolarizing the root, but this behavior should be capable of reversal under some conditions giving rise to results like those shown in figure 2; (b) it must be dependent on oxygen for the maintenance of its rectifying properties (fig. 9); (c) it must be either less efficient or absent in more basal segments of the root compared with the apical segment and especially the first millimeter of the root tip (figs. 1, 2, 3); (d) its effectiveness should decrease with duration of current flow (fig. 4), with larger currents (fig. 7), and at times with increasing frequency of application of current (curve A, fig. 5); and (e) its efficiency must be somewhat greater at lower temperatures especially in apical segments (fig. 8). In some respects it might seem that rectification is produced by the same structures responsible for the oxygen sensitive inherent PD but the disappearance of rectification in curve A, figure 4 between 40 and 60 minutes and in curve A, figure 5 between 90 and 130 minutes without any appreciable alteration of inherent potential makes this doubtful. Moreover, the fraction of total current flowing through the root that is rectified must be small enough not to be detected by the cathode ray oscillograph. Also no stimulation of the root with AC seems to occur even with relatively strong currents but on the basis of DC results (3) one might expect this to occur occasionally.

A stimulation of the root with AC can account for all of the observed results except the behavior of curve A, figure 4 and curve A, figure 5 men-

tioned in the paragraph above. It is difficult in the former case to understand how the observed potential can return to its original value during current flow when it is decreased during the first interval of flow. In the second case, the recovery of irritability at constant PD implies that there is no immediate relationship between the structures responsible for inherent potential and those which are thought to be broken down by the stimulating action of current flow (3). But with these exceptions all other observations agree in essential detail with the behavior of the roots stimulated with direct current (3). There seems to be no doubt but that DC applications may have one of two types of effect on the PD of the root and in some cases the change in potential observed may be an algebraic summation of the two.

The following points of agreement between the response of the root to applied AC and to DC stimulation may be cited: (a) the apical 5-mm. segment of a root is more readily stimulated than more basal segments, and when the latter are stimulated stronger currents are required (fig. 1); (b) stimulation by downward flow of DC (increase in the positivity of observed PD) occurs less commonly than stimulation by upward flow of current, hence the fact that curves like figure 2 appeared with only one root might be expected for this reason; (c) roots stimulated with AC can be again stimulated or polarized with DC of stimulating strength when applied before recovery is complete, the response to DC depending upon the amount of recovery from the AC stimulation (figs. 6, 7); (d) a lower temperature reduces the strength of current required to stimulate the apical segment of a root (fig. 8); and (e) the root cannot be stimulated in an atmosphere of hydrogen but can be stimulated in air (fig. 9).

It is probably true that there is no great choice between the two possible interpretations but it is the feeling of the writers that the latter is preferable because of the close agreement with the DC experiments. Work planned for the near future should make it possible, however, to base that choice on a sounder foundation.

Summary

1. The inherent potential of onion roots may be decreased by the application of alternating currents of sufficient strength. Only one exception was found and in this root the potential became more positive. No change is produced in the root PD unless "threshold" intensities are employed. These responses are more readily obtained in apical than in basal segments. In fact, when a stimulating current of an apical five millimeter segment is applied to each of the one millimeter segments, only the first millimeter of the root is stimulated and no change in PD is observed when it is not in the circuit.

2. As the duration of flow of a stimulating current increases from 5 to 60 or 120 seconds, the magnitude of root response becomes progressively smaller and may disappear. A return to the shorter flow of 5 seconds again

produces a large response, although not as great as initially. If a long recovery period is allowed the magnitude of the response may return to its former value.

3. If the times between applications of current are decreased, recovery of potential may not be complete, but the initial value after flow remains approximately the same. In some roots it is possible by this treatment to induce a "refractory" state, lasting for some time, during which no stimulation is possible. Eventually the root recovers its ability to respond.

4. When direct current of stimulating intensity is applied to a root whose potential has been lowered by alternating current of sufficient magnitude, it will no longer be stimulated but will only be polarized. The same thing will occur if the root has been made "refractory" with large alternating current.

5. The "threshold" for AC stimulation is less at low temperatures than at high temperatures. This effect is reversible and is more pronounced in apical segments than in basal segments.

6. A root which can be stimulated in air will no longer respond after a period in hydrogen, but will again respond when oxygen is admitted. This effect is perfectly reversible.

7. The results are interpreted as being due to the stimulating action of alternating current but the possibility of rectification by the root is discussed.

It is with pleasure that we acknowledge the technical assistance of Miss Athillen Jacobs so faithfully rendered during part of the investigation.

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LITERATURE CITED

1. AMLONG, H. U., and BÜNNING, E. Über elektromotorische Kräfte an elektrisch gereizten Wurzeln. Ber. d. bot. Ges. **52**: 445–457. 1934.

2. BERRY, L. JOE. Polarization potentials of short duration and their independence of the inherent potential in the onion root. In press.

3. ————, and HOYT, ROSALIE C. Polarization and stimulation of the onion root with direct current. Plant Physiol. **18**: 372–396. 1943.

4. BÜNNING, E. Elektrische Potentialänderungen an seismonastisch gereizten Staubfäder. Planta **22**: 251–268. 1934.

5. CLARK, W. G. Electrical polarity and auxin transport. Plant Physiol. **13**: 529–552. 1938.

6. METZNER, P. Über polare Leitfähigkeit lebender und toter Membranen. Ber. d. bot. Ges. **48**: 207–211. 1930.

QUANTITATIVE MEASUREMENT OF THE VELOCITY OF WATER ABSORPTION IN INDIVIDUAL ROOT HAIRS BY A MICROTECHNIQUE

HILDA F. ROSENE

(WITH SIX FIGURES)

POPESCO (11) credits the first observations on the absorption of solutes by roots to the work of MARCELLUS MALPIGHI in the seventeenth century. POPESCO writes that, in his work entitled *Anatome Plantarum* which appeared in 1675, MALPIGHI maintained that nutritive substances were absorbed by the roots at the level of the region of the root hairs. Since not all plants bear root hairs, MALPIGHI stated further that the entire epidermal surface functioned in absorption when root hairs were absent.

According to POPESCO, many early investigators following MALPIGHI were not in agreement with these conclusions: GREW in 1682 said that the roots absorbed water and nutritive substances from the earth through their tips, especially through the root cap; HALES in 1727 and DE LA BAISSE in 1733 supported GREW's contention stating in addition that the rôle of root hairs was secondary. Many of the observers in the beginning of the nineteenth century also believed that water and solutes were absorbed through the very tip of the root but in 1865 SACHS contended that the plant absorbed water from the earth by means of the root hairs, that the surface area of absorption was greatly increased by the presence of root hairs, and that volume absorption was proportionally increased. Subsequently, SCHWARZ calculated that the epidermal area in roots of corn was increased six times and in roots of peas twelve times when root hairs developed on these roots in moist air (10).

Such outstanding investigators of the early part of the twentieth century as JOST, MOLISCH, and PFEFFER also maintained that the principal rôle of water absorption in hairy roots was played by root hairs but several of their contemporaries disagreed, especially DE LAVISON (11) and COUPIN (11). In 1926 POPESCO published an account of his extensive experiments carried out to determine the absorbing region of the root. From results based on indirect methods he concluded that the presence or absence of root hairs did not perceptibly influence the absorption of solutes and did not modify the limits of the absorbing region: "La présence ou l'absence de poils radicaux n'influence pas sensiblement le phénomène d'absorption des solutions et ne modifie pas les limites de la région absorbante" (11, p. 136).

More recent investigations have been made by HÖHN (7) and by SIERP and BREWIG (15). HÖHN concluded that it was very probable that root hairs were not able to increase the volume of water absorbed in water cultures and that the presence or absence of root hairs changed nothing in the quantitative conditions of water absorption in the various root zones. He

based his conclusions on duplicate potometric experiments with hairbearing and hairless roots of seedlings (*Zea mays, Vicia faba,* and *Tradescantia fluminensis*). SIERP and BREWIG, on the other hand, found that the region of greatest water absorption in *Vicia faba* roots was that section fifteen to eighty millimeters from the apex which ordinarily possesses root hairs; whereas the zone without hairs showed an uptake of an insignificant quantity. Their measurements were not made on the same roots which possessed hairs; observations were made on regions of roots which corresponded to the root hair regions of other roots.

Modern texts in botany and more specifically, in plant physiology, written almost three hundred years after MALPIGHI's publications, are in general agreement that the presence of root hairs increases the absorbing surface of roots to a marked extent and that "nearly all water and salts which enter the plant from the soil do so through root hairs" (14) which are in intimate contact with the soil particles. Although investigations dealing with the problem of water absorption in roots have been carried out for centuries, no successful attempt appears to have been made to prove by direct means that the root hair *per se* is an absorbing mechanism. Considering the vast amount of literature on many aspects of the general physiology of root hairs, it is surprising to find an absence of conclusive data on the question of whether or not the root hair does actually function as an absorbing mechanism. The numerous investigators who worked on the problem distinguished between regions with and without root hairs and between hairbearing and hairless roots, but they did not distinguish between absorption through the surfaces of a root hair extension of the epidermal cell and absorption through the surface of an epidermal cell without such an extension. Where root hairs are present, absorption might take place through: (a), the surface of the root hair extension; (b), the epidermal area at the base of the root hair extension; (c), the epidermal cells which have no root hair extension and which are located between the hairbearing cells; (d), the hairless epidermal cells distal or proximal to the hairbearing regions.

The problem of absorption of water and solutes by root hairs is one which involves a *single* hair; in a fundamental sense, therefore, it is a problem in cell dynamics since the epidermal cell with its root hair extension is a single cell. The superficial position of the hair cells on the surface of the root, their relatively rapid development and growth, the absence of chlorophyll, and the fact that they are uninucleate make them very favorable material for numerous studies in cell dynamics.

No doubt the chief reason that no conclusive investigation has been made to determine the rôle of the root hair as an absorbing mechanism results from the difficulties of the technique involved in a realm of such small dimensions. As far as the author knows, the present study is the first to furnish quantitative data on the question of whether or not root hairs function in the absorption of water. The technique is a micromodification of that previously used by the author in studies on water absorption in the

onion root (12). This micromodification permits observations to be made on a single root hair.

Micropotometers were constructed from glass capillaries and fastened in a horizontal position to a vertical support. Since the hairbearing epidermal cell elongates perpendicularly to the longitudinal axis of the root, the hair extension was readily inserted into a single micropotometer. Accurate measurements of length were possible because the horizon of the root at the base of each hair and the terminus at the apex were definite. Determination of the surface area exposed to the solution in a micropoto·, was simplified by the fact that the tubular hair extension is unif· diameter and ends in a blunt dome-shaped apex. The data repo┌ ⌐ this investigation were obtained from the root hairs of radish seedlings (*Raphanus sativus*).

Apparatus

Plates of thin glass, free from aberration, were fastened together with de Khotinsky cement to form the rectangular chamber N shown in figure 1. It is 5 cm. square and 11.5 cm. high. It fits into a depression, A, cut into the base, B, made of transite which had been immersed in hot paraffin to make it water tight. The base, B, which is supported by the rod, S, has four perforations each fitted with a guard made from glass tubing. Through these guards pass, respectively, a thermometer holder, C, a movable glass rod "window wiper," D, a movable root holder shaft, I, and a movable root "adjustor," F. The window wiper consists of a glass rod with a double bend; a piece of rubber tubing placed over the second bend serves to remove moisture from the glass wall facing the horizontal microscope.

The seedling holder consists of a small saddle, E, attached to a small watch wheel in the frame, H, above the saddle. The saddle with the attached seedling is moved on its axis through the action of two watch wheels which are operated by moving the milled head, G, of a rod attached to one of them; this rod moves in the shaft, I, which has a collar, J, to support a clamp; by this means the root may be rotated on its axis. The wheels are thoroughly greased with vaseline and covered by a small rectangular box cut from a piece of lucite rod. The glass root adjustor consists of a section of rod with a double bend at the top end, the tip of which was drawn out to a delicate projection with a blunt curved terminus. The root adjustor was used to place the seedling in a suitable position in the seedling holder.

The micropotometer support, K, is a glass rod pulled out to a very small diameter at the end on which two micropotometers, M, were attached with de Khotinsky cement. It is inserted through the glass guard, L, at the top of the chamber. This glass guard forms a rim 15 mm. in height attached to the edge of a hole 25 mm. in diameter cut through the chamber top.

The glass guards are. necessary to permit freedom of movement with horizontal or vertical displacement of the rods which pass through the various openings. In order to provide moisture- and air-tight seals with suffi-

cient movement at the perforations through which the rods pass, latex rubber finger cots are used.

The pair of micropotometers, M, consists of glass capillaries made from carefully selected Pyrex tubing. Short lengths of tubing were pulled into fine capillaries of uniform bore and sections were cut from the capillaries to make the micropotometers. One end of each section was cautiously fired to constrict the inside diameter. This constricted end will be referred to as ''.~ proximal opening and the other as the distal end. This constriction was

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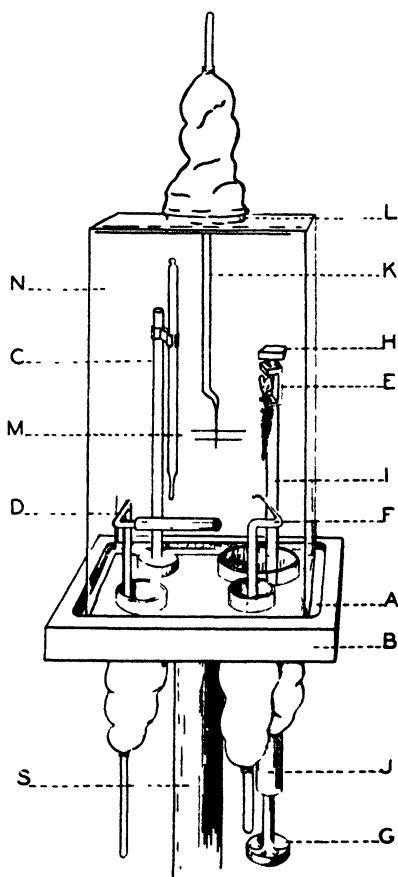
FIG. 1. Experimental chamber. See text for description.

necessary in order to hold the meniscus at the proximal end of the micropotometer capillary. Micropotometers of different inside diameters were used in different experiments but each pair was made from adjacent sections of a pulled capillary in order to match them. Dimensions of a typical micropotometer into which a single hair is inserted are: length, 10 mm.; inside diameter, 58.9 microns; diameter of opening at constricted end, 34.2 microns. The micropotometer capillaries were too fragile to be handled by forceps. Each was handled by placing a minute drop of water on it and taking advantage of surface tension forces.

One micropotometer of each pair served as a control. The pair of micropotometers were cemented to the potometer support in such a way that the micropotometer used to test absorption extended beyond the control and was, therefore, nearer the hairs. With this arrangement it was possible to insert a single root hair into one micropotometer without permitting other hairs to come into contact with the control capillary.

The glass rod, K, supporting the micropotometers and the collar, J, of the root holder support are each clamped to a micromanipulator not shown in the figure. An individual root hair is inserted into the proximal end of the micropotometer by adjustments made with the micromanipulators. All adjustments of the micromanipulators are made with the aid of a horizontal microscope fitted with a Filar micrometer. By this means very delicate adjustments of the positions of either the root holder or micropotometers can be made without mechanical injury to the root hair.

The apparatus used, including the horizontal microscope, was placed within an auxiliary chamber with a wide opening in front covered by a drop curtain of black sateen. This afforded an opportunity to permit development and growth of hairs on a seedling within the experimental chamber in the dark while other work necessitating light could be carried on in the laboratory at the same time.

An indirect source of light is produced by a beam from a light placed at a distance of four feet from the wall of the auxiliary chamber. The beam passes through a flat flask containing a solution of copper sulphate, and penetrates the auxiliary chamber through a small hole. The beam is reflected from a concave mirror which was clamped to a micromanipulator. This provided a concentrated light which could be accurately placed on any region of the micropotometers, hairs, or the seedling. A fluorescent lamp placed within the auxiliary chamber provided a direct source of light. No precaution was taken to use a ruby light since JEFFS (8) found, using a wide range of candle powers for various periods of time, that the root hairs of *Raphanus sativus* showed no light-growth reaction. In most instances, in the present work, the fluorescent lamp was used as the light source. It was usually turned on only when making measurements or setting up an experiment.

Procedure

The seeds were soaked for 24 hours and then germinated on wet filter paper in Petri dishes placed in the dark. The experiments were conducted in a fair-sized basement room with thick walls. It was possible to maintain a practically constant temperature when the room was not used for other experimental work involving the use of lights, etc.

The experimental chamber was partly lined with strips of filter paper; the depression (A, fig. 1) in the transite base and the finger cots attached to the guards in the base were filled with distilled water. In this way the floor of the experimental chamber was flooded with water, and the filter

paper strips were wet since they extended into the water when the chamber was placed in position.

Seedlings were placed in the experimental chamber either a few hours preceding the taking of measurements or the night before. In either case, data were obtained from hairs which developed in the moist atmosphere within the experimental chamber. The general procedure is as follows: the micropotometer support and the attached finger cot are removed from the top of the chamber and fastened in a clamp on a nearby stand. A rubber stopper is fitted temporarily into the guard on top of the chamber. With the experimental chamber in the left hand, a seedling which has a short root is gently removed with forceps from a Petri dish and placed in the root holder where it is secured with a small amount of petrolatum. The chamber is quickly replaced and the glass root adjustor is manipulated from the outside to make necessary adjustments of the seedling position; it is held in an upright position with respect to gravity. The micropotometers are filled and the supporting glass rod clamped to a micromanipulator by means of which it is lowered into the chamber having removed the rubber stopper from its top. The preparation is left undisturbed until readings are to be made. The micropotometers are then moved into a position in close proximity to the hair selected for observation. With the aid of the microscope the hair is threaded into the proximal opening of the upper micropotometer by (a) moving the micropotometer over the root hair or by (b) moving the root holder and thus the hair into the micropotometer which in this case is held in a fixed position. This was possible without permitting the hair to come in contact with the glass surface and thus producing mechanical injury. Difficulty was experienced when the hairs were in close proximity, especially if the micropotometers touched adjacent hairs; root hairs are "sticky" and will adhere firmly to the outside surface of the capillaries.

A special technique had to be developed to clean and refill the fragile micropotometers. Solutions were drawn into the capillaries by placing the proximal end in contact with the liquid. Solutions were forced out of the micropotometer capillaries by applying a fine jet of air or water at the distal end. Much care was exercised in cleaning and refilling the flexible micropotometers immediately following an experiment.

Change in length of the water column within each micropotometer was determined by making consecutive measurements of the movement of the distal menisci with the Filar micrometer. A number of experiments were carried out to determine whether or not movement of the water menisci in the micropotometers occurred in the absence of (a) any seedling in the experimental chamber and (b) in the absence of a root hair in the upper micropotometer when a seedling was growing within the experimental chamber. As will be shown later, movement of the distal menisci in the micropotometers usually occurred in the absence of root hairs or of any seedling within the chamber. Such movement indicated that under the given conditions either evaporation occurred, or temperature changes produced variations in the length of the water column in the micropotometers.

Since no constant temperature, constant humidity room was available, it was impossible to completely control these factors. Although movement of the distal menisci in both micropotometers occurred, the rate of movement in the upper capillary with all or part of the root hair immersed was significantly greater than the rate of the corresponding moving meniscus of the control capillary. The factors responsible for evaporation were unknown. A possible source of evaporation from the chamber itself was the upper latex finger cot around the glass guard, L, of figure 1. Inverting it and filling it with water, however, made an effective seal but movement of the distal menisci in both micropotometers continued. Various small inequalities in vapor tension due to localized unstable conditions on different small surfaces might be responsible for evaporation. It may be that evaporation occurred from the surface of the meniscus at the constricted proximal opening as a result of a localized high vapor tension over its small curved surface as compared to a relatively lower vapor tension over the plane surface of the larger body of water flooding the chamber floor. According to ADAM (1) "the vapour pressure over a convex surface is greater than that over a plane; and over a concave surface it is less." He cites data obtained by THOMÄ (16) who showed that vapor pressure is greater with a smaller surface. The area of the proximal opening of the upper micropotometer, into which the hair was inserted, was greater than that of the lower micropotometer, which served as a control, by the cross-sectional area of a typical root hair. In this way the areas of the water-vapor interfaces of the proximal openings were comparable although their shapes were different.

In order to determine volume absorption during unit time by a given region of an individual hair cell, the data obtained from volume displacement indicated by movement of the distal meniscus in the root hair micropotometer during each interval were corrected for evaporation by subtracting data obtained from corresponding measurements of any movement of the control meniscus. Calculations of the velocity of water absorption were based upon the calculated volume absorption during unit time and measurements of the length and diameter of the immersed region. Although the apex of each hair was dome-shaped, no corrections were made for the variation in diameter at the extreme tip; the slight variation in area due to curvature was considered negligible in the present experiments.

The technique was subject to errors inherent in microscopic measurements. Errors due to readings were, of course, largest when the rate of movement of the distal menisci was slow since it was difficult to determine precisely a linear displacement of less than 2 microns. Percentage error due to readings, therefore, would depend upon the total linear displacement during any one interval; such displacement would be greater with capillaries of smaller diameter, other things being equal. An example of a relatively large difference in linear displacement during a ten-minute interval is 226 microns in the root hair micropotometer capillary and 16 microns in the control capillary. Similarly, errors in the determination of surface areas

would be proportionately greater in root hairs of comparatively small diameters.

An analysis of the tap water used in the experiments showed the following in p.p.m.:

Total dissolved solids 143

Ca 13.0	CO_3 12	Cl 30.0
Mg 4.5	HCO_3 37	F 0.2
Na 23.0	SO_4 27	NO_3 0.6

Measurements of hydrogen ion concentration showed a pH of 8.2 at 25° C. The osmotic pressure calculated from the freezing point lowering was 0.122 atm. at 25° C.

Results

The diagram below the curves in figure 2 shows a root hair inserted through the constricted proximal opening (p) of a capillary micropotometer, the distal end (d) of which is also illustrated. The dotted line (m) indicates the position of the distal water meniscus at the beginning of an experiment and the arrow the direction of its linear displacement during absorption of water through the surface of the root hair extending into the micropotometer. "Volume movement" or "volume displacement" of the distal meniscus in the control capillary and in the micropotometer containing the root hair, during unit time, was determined by multiplying the linear displacement of the distal meniscus in each by its corresponding cross-sectional area, neglecting curvature of the meniscus. Curve 1, figure 2, shows the average "volume movement" (cu. mm./min.) indicated by the distal meniscus in a capillary micropotometer containing a root hair almost totally immersed during six consecutive ten-minute intervals; curve 2 shows the "volume movement" in the corresponding control capillary. The difference in magnitude of the two curves indicates that a relatively large volume of water was steadily absorbed by the hairbearing epidermal cell through its immersed surface which was 0.0478 square millimeters. This difference in magnitude which gives the calculated volume of water absorbed in unit interval of time during each ten-minute interval is plotted in curve 3 (ordinate on left). Curve 2 shows that a small non-uniform rate of "evaporation" was manifested by the control; the deviations, however, fall within the range which might be expected due to limitations in taking measurements. But the deviations from a constant volume absorption during each ten-minute interval as indicated by curve 3 (fig. 2) are greater than those which might be expected from experimental error; they point to a drift in the average velocity of absorption with time. These variations in the volume of water absorbed by the hair cell and "evaporation" from the control with time were evidently independent of observed temperature change. Curve 4, figure 2, was plotted from calculations obtained by dividing the calculated volume of water absorbed during unit time through the surface of the root hair exposed to the solution by the area of this sur-

face determined from measurements of the length and diameter of the immersed region. It therefore shows the average velocity of absorption of water through the immersed region of the hair cell during each ten-minute interval.
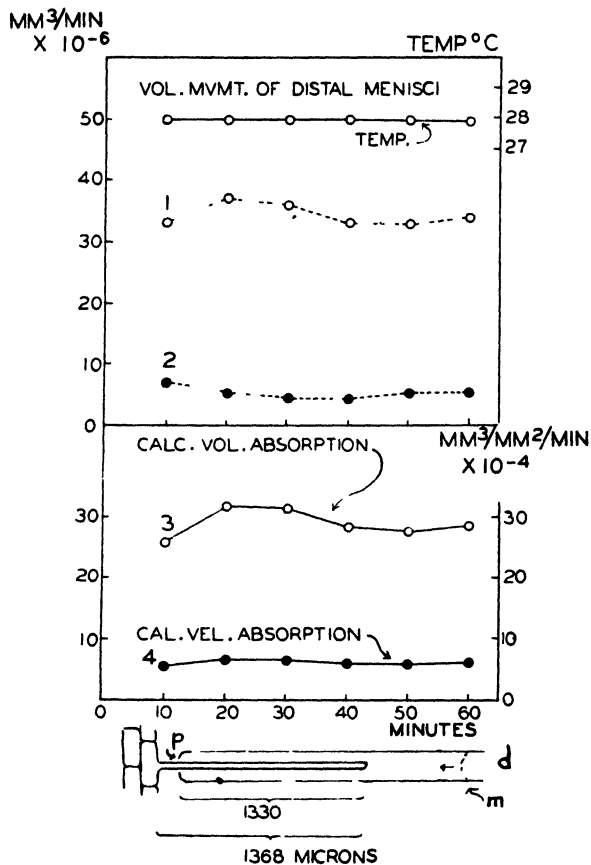
FIG. 2. Water absorption by a root hair almost totally immersed. Data obtained from a seedling which had been placed in the experimental chamber the preceding evening, the hair under observation having appeared during the night, probably during the early morning hours. The seedling was selected from a Petri dish into which seeds had been placed four days earlier. The diagram below the curves shows the length of root hair immersed and the total length of the hair; p indicates the proximal and d the distal end of the micropotometer with the middle section not showing; m indicates the distal meniscus of the solution. Curves 1 and 2 show the average volume displacement indicated by the distal menisci in the root hair micropotometer and the control, respectively, during unit time (ordinate on left). Curve 3 shows the calculated volume of water absorbed by the hair in unit time (ordinate on left) and curve 4 the calculated velocity of absorption through unit surface (ordinate on right). Temperature readings were made at ten-minute intervals and the average of two successive readings plotted as the temperature during the intervals.

It is important to note that in figure 2 and in all subsequent figures the ordinate for the velocity of absorption is on the right and that its scale is

different from that on the left.   This was done in order to plot all the curves from a single experiment on one figure.   The scale selected was a matter of convenience; the fact that curve 4 is nearer the base line than the other curves has no other significance.

The curves of figure 3 show typical results obtained during experiments of several hours duration.   The seedling was placed in the experimental chamber 30 hours after the seeds had been put into a Petri dish to germinate. There were no hairs on the short root when the seedling was placed in the
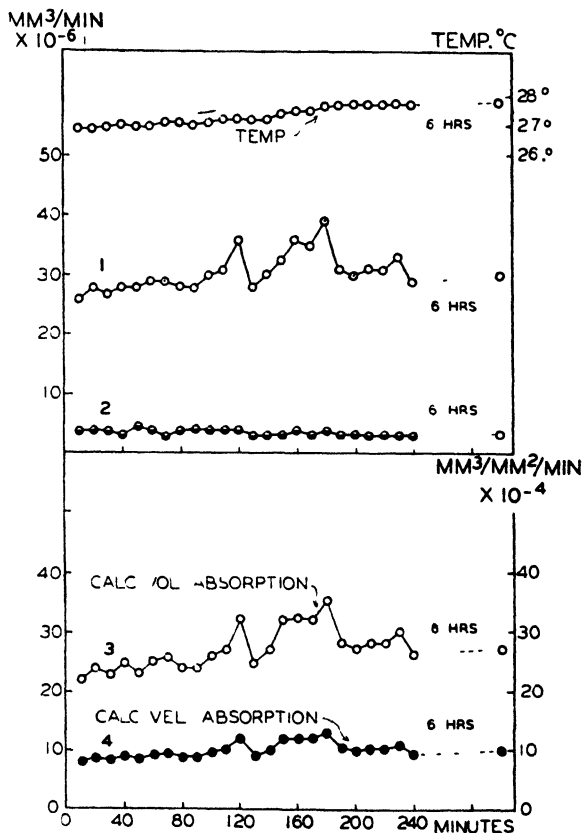
FIG. 3.   Results obtained during a relatively long period of immersion.   Total length of root hair, 798 μ; length of immersed region, 570 μ; area of surface exposed to water, 0.027 square millimeters.   See legend of figure 2 for explanation of the curves.   Observations were made at ten minute intervals for four hours and finally at the end of a 6-hour interval.

chamber the night preceding the experiment.   A young hair was selected for observation the next morning.   It did not grow during its relatively long period of immersion but subsequent growth occurred in the moist chamber air during the following night.   The average rate of evaporation from the control capillary was comparatively low during all ten-minute intervals throughout the first four hours of observation (cf. curve 2, fig. 3, with curve
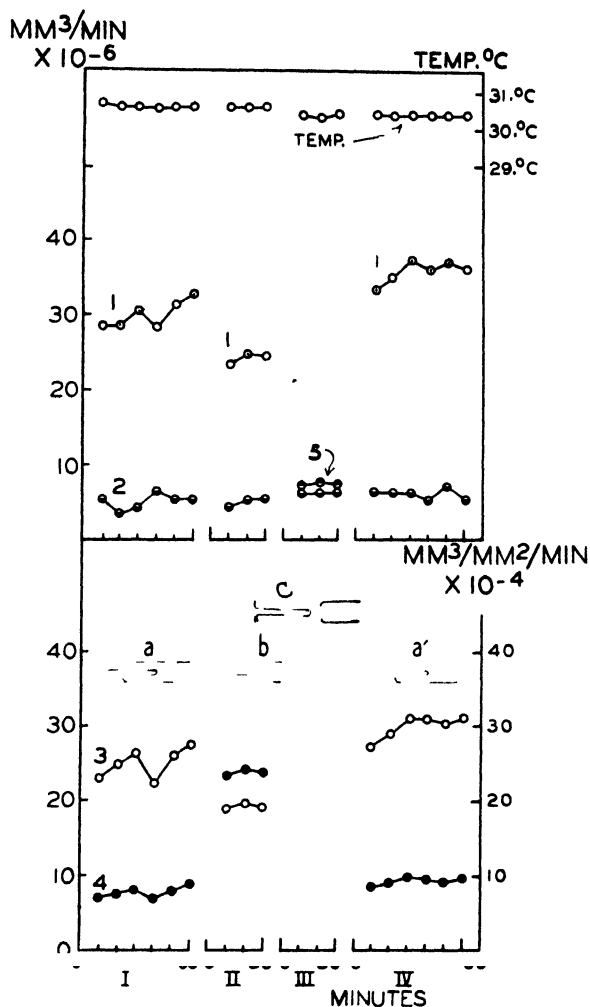
FIG. 4. Absorption when different lengths of a single root hair are exposed during different time periods. The diagrams above curve 3 show the relative lengths of root hair immersed during different consecutive time periods which are numbered in Roman numerals below the base line. The breaks in each curve indicate the time during which the position of the hair in the micropotometer was changed. The different parts of curve 1 are indicated by circles with a dot in the center; curve 2, by circles with lower half black; curve 3, by white circles; curve 4 by black circles. See legend for figure 2 for further explanation of curves 1, 2, 3, and 4. Curve 5 shows volume displacement indicated by the distal meniscus of the root hair micropotometer during unit time after the root hair was withdrawn; in this instance both capillary micropotometers served as controls.

⊙—⊙—⊙ = volume displacement in root hair cap.

●—●—● = volume displacement in control.

○—○—○ = calculated volume absorption.

= calculated velocity absorption.

2, fig. 2). The average rate of evaporation during the subsequent 6-hour interval was also low. No marked fluctuations in the rate of evaporation were observed. On the other hand, curve 1 shows not only an overall drift of volume displacement indicated by the distal meniscus in the root hair micropotometer but also pronounced deviations from interval to interval, especially after the second hour. The oscillations in curves 1, 3, and 4 are outside the range expected from experimental error; those of curve 2, within this range. Temperature variations also occurred; the temperature increased from an initial value of 26.9° to 28.75° C. at the end of 190 minutes; it then remained constant. The slopes of curves 3 and 4 are, in general, similar to the slope of the temperature curve, indicating a possible temperature effect on the absorbing mechanism of the root hair; the slope of curve 2 indicates a comparative absence of a temperature effect on the length of the water column in the control micropotometer and the rate of evaporation. Curve 4 shows that a fairly high average velocity of absorption of water was maintained throughout the ten hours of immersion.

The curves in figure 4 were obtained from a root hair on a seedling placed in the chamber in the morning. Within a half hour of transference new root hairs appeared. Six hours later one of these hairs was selected to determine the velocity of water absorption through different surface regions along its longitudinal axis. The length of the hair was 1140 microns. Four different time periods are indicated by Roman numerals on the base line in figure 4. The initial temperature was 30.6° C.; it dropped to 30.2° during the third period, then rose to 30.4° in the fourth period. During the first period, which was 60 minutes in duration, observations were made when the distal region immersed was 684 microns in length and the area 0.0324 square millimeters as illustrated in diagram a, figure 4. The root hair was then partially withdrawn from the micropotometer tube until the length exposed was 171 microns and the area 0.0081 square millimeters (diagram b, fig. 4). Observations were again made during a second period which was only thirty minutes in duration. During a third period of thirty minutes duration, the root hair was completely withdrawn as indicated in diagram c in order to determine whether or not the distal meniscus in the root hair micropotometer would continue to move in the absence of the root hair. Finally, preceding a fourth period, the root was again moved (diagram a′) to immerse the same hair region which was immersed during the first time period. Each curve in figure 4 shows breaks; each break indicates the time during which the position of the root hair was changed; the various parts of each curve have the same type of circles and represent data obtained during the corresponding time periods indicated on the base line.

As in the preceding experiments, there was a relatively large difference between the volume displacement indicated by the distal menisci in the control (curve 2) and the root hair (curve 1) micropotometers when the root hair was exposed to water during intervals I, II, and III. When, how-

ever, the root hair was withdrawn (curve 5) the difference was slight. A comparison of the different parts of curve 3 shows that during period II when the hair was in position b and the area exposed was 0.0081 square millimeters, the calculated volume of water absorbed during unit time was less than that absorbed during unit time in periods I and IV when the root hair was in position a or a' and the area exposed was 0.0324 square millimeters. Although the area exposed, during periods I and IV, was four times greater than that exposed during period II, no such marked difference was observed in volume intake during unit time. This may be partially accounted for by examining curve 4, figure 4, which shows the average velocity of absorption of the surface exposed during the different intervals.

A comparison of the different parts of curve 4, figure 4, shows that *when the length of root hair in the micropotometer was reduced to one-fourth* (from 648 microns in position a, period I, to 171 microns in position b, period II), *the average rate of water intake through unit surface area was tripled.* When, during period IV, the length was again increased to 648 microns (in position a', period IV), the average velocity of absorption manifested by each unit of surface decreased to its initial value. This indicates that the average velocity of absorption per unit area at the tip of the root hair is much greater than that per unit area in regions relatively more proximal under the particular conditions of the experiment. It does not, however, show whether or not the same velocity obtained at the tip when it was in position a or a' as when it was in position b. Although the temperature was constant during most of the first and all of the second period, variations appeared in the velocity of intake and the volume absorbed during unit time.

The curves of figures 5 and 6 were also obtained from individual hairs by varying the region observed during different time periods. Observations were made on water absorption by three different root hairs (A, B, C), each on a different root. The three experiments were run on different days; in each the seedling was placed in the chamber the preceding night. Root hair A was 874 microns long; the region inserted into the micropotometer during time periods I and III was 722 microns in length and 0.019 square millimeters in area (diagrams a and a'); during period II the distal half of this area (diagram b) was exposed to water. Root hair B was practically twice as long (1710 microns) as root hair A; half of its length (immersed area: 0.031 sq. mm.) was inserted during periods I and III (diagrams a and a'); one-fourth of its length (immersed area: 0.0155 sq. mm.) during period II (diagram b). An inspection of curves 3 and 4 for each root hair shows that although the calculated volumes of water absorbed per unit interval of time (curve 3) were reduced during period II when the area of absorption was reduced by one-half, the average velocity of absorption (curve 4) was greater than that observed during periods I and III. During period II, almost one-half of the total area of root hair A was exposed to water and one-fourth of the total area of root hair B. It is interesting that in root

hair B where a proportionately smaller region of the distal end of the hair was exposed, the volume decrease in absorption per unit time was less, and the increase in velocity per unit area was greater, than that observed in root hair A; although in period II of each experiment the area exposed had been reduced by one-half. The significance of this fact is more apparent when the variations in the curves of root hair C (fig. 6) are compared.

Root hair C (fig. 6) was 1387 microns in length; the regions immersed in the micropotometer during periods I, II, and III were respectively 760, 380, and 190 microns in length and 0.0224, 0.0112, and 0.0056 square millimeters
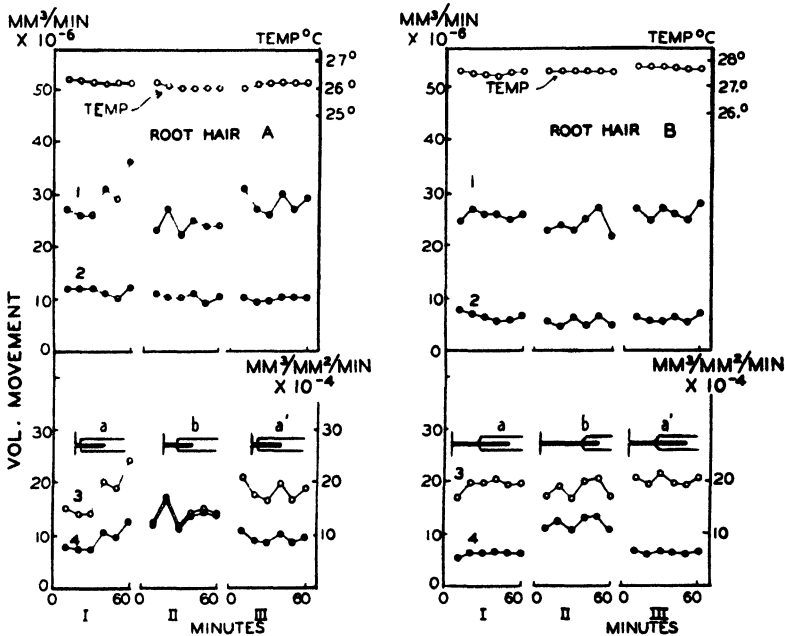
FIG. 5. Absorption by different regions of different root hairs. Curves for two different root hairs (A, B), each on a different seedling, are given. See legends of figures 2 and 4 for explanation of curves.

in area in the respective positions indicated by diagrams a, b, and c. The root hair was then completely withdrawn as indicated in diagram d, period IV, and observations were made on both micropotometers as controls (curves 2 and 5, period IV). Subsequently, observations were made during periods V, VI, and VII when a reverse procedure was used, increasing the area of exposure at stated intervals. The curves obtained during periods III and V are, therefore, comparable as are the curves for periods II and VI, and I and VII—since the same regions were exposed although at different times. The average total volume of water absorbed (curve 3) during unit time is greatest, and the average velocity of absorption (curve 4) is least, during periods I and VII when the areas of exposure were greatest. With diminishing area, a decrease in the average volume of absorption per unit

time and increase in the average velocity of absorption appeared; the reverse was true with increasing area.

Although diminution of area occurred within the micropotometer when the root hair was partially withdrawn, the actual absorbing area might have been greater than the observed diminished area due to a possible residual film of water along the sides of the hair when it was partially withdrawn. If so, the observed increase in velocity was only an apparent increase. Attention, however, is called to the fact that in the experiments from which the curves in figure 6, root hair C, and figure 4 were obtained, the hair was completely withdrawn for over an hour and then reinserted by increasing the area at specified intervals. In further experiments, not reported, similar results were obtained. In the reverse procedure, opposite results would be expected if the observed area were less than the actual area when the hair
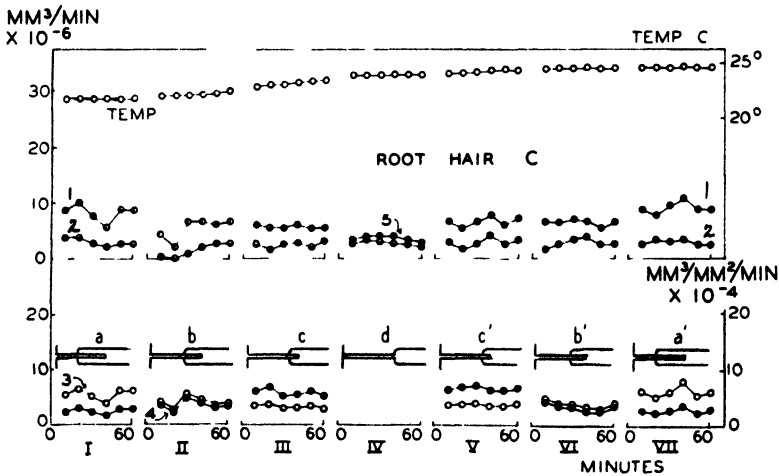
FIG. 6. Absorption by different regions of an individual root hair. See legends of figures 2 and 4 for explanation of curves.

was withdrawn. Hence, the data obtained in the experiments during which the area of immersion was varied in different periods show that when the area of exposure was confined to the relatively distal end of the hair, the average velocity of absorption per unit area was actually greater than that manifested when the area of exposure included not only the distal end but proximal regions also. This indicates either that the average velocity of water entry in the region at the tip was markedly different when the area of exposure was varied and that this difference was due to changes produced by varying the area; or that varying the area did not produce the observed increase in average velocity of absorption, and that this increase indicates a distinct polarity in the distribution of velocity of absorption along the longitudinal axis of the individual hair cell, higher velocities occurring in relatively more distal regions. A final test to determine whether or not such a polarity exists in the type of hairs studied would be simultaneous measurement of water absorption in different regions of an individual hair

employing a technique similar to that used by the author in work on the onion root (**13**). It might also be that a compensating mechanism and a distinct functional polarity are both present.

Discussion

For the first time, after many decades of conflicting opinion and speculation beginning with the early microscopists, quantitative data have been obtained in support of the many writers who have maintained without direct experimental evidence that root hairs function in the process of water absorption. It has been claimed by many workers that root hairs play a primary rôle in absorption of water from the soil. The increase in surface area due to the presence of root hairs has been determined by numerous investigators. But the question as to what extent root hairs will facilitate absorption by increasing the surface exposed to the external solution is one which cannot be answered by mere determination of the surface area; it is necessary in addition to determine the velocities of absorption of both hairbearing and adjacent hairless epidermal cells. In the onion root (**12**) the comparatively larger share of water intake by relatively older root regions expresses itself as a larger capacity for absorption. Relatively high velocities of absorption in the hairless epidermal cells could compensate for lower rates over a larger area of hair surfaces. The new technique combined with that used by the author in onion root studies provides, for the first time, a method for obtaining quantitative data on this question.

Since no measurements of water absorption by the hairless epidermal cells of radish roots are as yet available, it is interesting to compare the velocities of absorption of hairless epidermal cells of the onion root with results obtained on the hair cells in this investigation. Although, in a strict sense, a comparison is not valid since different types of plants and different solutions were used, the comparison indicates that the velocity of absorption by the hair cell falls within the range of velocities exhibited by hairless cells. In the present investigation the maximum velocity observed in a hair cell of the radish root was 31×10^{-4} cu. mm./sq. mm./min. The significance of this value as a maximum remains to be shown as the work on hair absorption has only started. In the onion root the average velocities of absorption from HOAGLAND's solution by the hairless cells in the vicinity of the regions of elongation and meristem are lower than this value; in regions 45 mm. from the root tip, the velocities of absorption of distilled water may be as high as 84×10^{-4} cu. mm./sq. mm./min. or as low as 4×10^{-4} cu. mm./sq. mm./min. (**13**). Minimum velocities observed in hair cells were 2 to 3×10^{-4} cu. mm./sq. mm./min.

Raphanus sativus is one of the 87 species of plants reported by FARR (**5**) which form hairs in water. Although root hairs develop on radish seedlings in either tap water or moist air, no elongation of an individual hair was observed during the period of immersion in the experiments reported in this paper. Elongation, however, was resumed after the period

of immersion, showing that the inhibition was reversible. This phenomenon is in agreement with observations by other investigators who have noted that reversible inhibition of elongation of root hairs frequently occurs when a seedling is transferred from one medium to another (**6, 17**). The experimental chamber itself served as an excellent culture chamber for the individual seedlings which formed an abundance of parallel straight hairs with uniform diameter at right angles to the root. No abnormalities of growth were observed and rupturing of hair cells rarely occurred except as a result of mechanical injury. There was an absence of visible expansion or contraction of the root hair within the limits of micrometer readings.

The experiments furnish strong evidence for unequal absorption in different areas of the individual cell under the given conditions. This shows that a comparison on the basis of surface is of great importance. It might be that there is also a functional polarity of water absorption along the longitudinal axis of the root hair when totally immersed, with the greatest velocity of absorption at the apical end. Further experimentation is necessary to establish this as a fact. The phenomenon of cellular polarity has a special interest to the general physiologist; no data have been reported heretofore which definitely show that different minute regions of a single cell absorb water at different rates. CHAMBERS and KERR (**2**) found that the wall of the root hair (*Limnobium spongia*) in both living and dead cells was more freely permeable to ammonia at the tip than elsewhere; CHAMBERS and KEMPTON (**3**) have reported a characteristic polarity of the proximal tubules of the chick embryo; the cells absorb phenol red from the external medium on one side and transfer it through the cell and out into the lumen of the tubule on the other side. LUND (**9**) found a distinct polarity in filamentous algal cells with respect to the distribution of bioelectric potentials along the longitudinal axis; the highest E.M.F. appeared at the apical growing point of the apical cell and in the apical regions of adjacent cells.

The data do not show which factors are responsible for the observed differences in velocity exhibited by different regions; on the one hand, they might be due to intracellular differences in metabolic energy or osmotic energy; on the other hand, they might be due to physical characteristics of the limiting membranes. It will be of extreme interest and importance to determine the effect of the presence or absence of oxygen, and the effect of poisoning with narcotics, cyanide, and other respiratory poisons. The technique employed in the present experiments provides for the passage of gases through the sealed chamber and for the absorption of different substances from the micropotometers. It therefore serves as a unique tool to study the factors which regulate the entry of water into the single hair cell.

Of particular interest in connection with determination of the physical or metabolic factors responsible for the differences in velocity of absorption over the surface of an individual root hair observed in the present work is the presence of the nucleus at or near the tip of the radish hair and the

differences in the structure of the cell wall. The structure of the radish root hair has received some attention from CORMACK (4) who worked chiefly with varieties of Chinese cabbage but used other plants to test the general applicability of his conclusions. He reports that the wall of a normal hair has a cellulose and a pectic layer continuous with corresponding layers in the epidermal cell; that while the root hair is growing the cellulose layer is soft and easily hydrolyzed and gives every indication of being uniform over the whole surface; that the pectic layer on the sides of the hair is of firm calcium pectate; and the same is true of the parent cell walls. On the dome-shaped tip the layer is composed either of pectic acid or of a softer calcium pectate. In the mature hair the cellulose wall is less easily hydrolyzed and the surface is covered by a layer of calcium pectate of the firmer type. A comparison of the intake of water by young root hairs with that of old root hairs may throw light on numerous problems and since the root hairs of certain water plants (2) have nuclei at the base of the hair, they can serve as material for comparative studies on the relation of the nucleus to polarity.

Of further interest is the elucidation of the rôle played by cortical cells adjacent to the hairbearing epidermal cell as well as cells beyond the cortex. In order that continuous water entry might take place in a cell which does not increase in volume there must be a continuous transfer of water to adjacent cells. The speed of absorption may be determined by resistance to passage of water offered by such adjacent cells. The author has developed a method to study this problem.

The technique has a peculiarly significant relation to problems of soil science. Individual micropotometers simulate capillary passages in the soil and this method provides an approach to the problem of determining why different plants do not avail themselves equally of soil moisture.

Summary

1. A microtechnique which may be applied to a quantitative study of the absorption of water and solutes by an individual root hair cell is described.

2. The results furnish for the first time quantitative data in support of the many investigators who have maintained, without direct experimental evidence, that root hairs function in the process of water absorption by higher plants.

3. Measurements were made on the absorption of tap water by individual root hairs of young radish (*Raphanus sativus*) seedlings. The maximum average velocity of absorption observed under the particular conditions described was 31×10^4 cu. mm./sq. mm./min.; the minimum was 2×10^{-4} cu. mm./sq. mm./min.

4. The data furnish strong evidence for unequal absorption in different areas of a single hairbearing epidermal cell under the particular conditions described.

5. Attention is called to how the microtechnique described furnishes a unique tool to study factors which regulate the entry of water and solutes into a single cell and in higher plants.

Grateful acknowledgement is made to my technical assistant A. A. HORAK for his aid in this investigation and to PROFESSOR E. P. SCHOCH, of the Bureau of Industrial Chemistry, for tap water analysis.

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LITERATURE CITED

1. ADAM, N. K.   The physics and chemistry of surfaces. p. 13.   The Clarendon Press, Oxford. 1938.
2. CHAMBERS, R., and KERR, T.   Intracellular hydrion concentration studies. VIII. Cytoplasm and vacuole of *Limnobium* root-hair cells. Jour. Cell. & Comp. Physiol. **2**: 105–119. 1932.
3. ——————— and KEMPTON, RUDOLPH T.   Indications of function of the chick mesonephros in tissue culture with phenol red. Jour. Cell. Comp. Physiol. **3**: 131–167. 1933.
4. CORMACK, R. G. H.   Investigations on development of root hairs. New Phytol. **34**: 30–54. 1935.
5. FARR, C. H.   The formation of root hairs in water. Proc. Iowa Acad. Sci. **32**: 157–165. 1925.
6. ———————.   Studies on the growth of root hairs in solutions. II. The effects of concentrations of calcium nitrate. Amer. Jour. Bot. **14**: 497–515. 1927.
7. HÖHN, K.   Die Bedeutung der Wurzelhaare für die Wasseraufnahme der Pflanzen. Zeitschr. f. Bot. **27**: 529–564. 1934.
8. JEFFS, R. E.   The elongation of root hairs as affected by light and temperature. Amer. Jour. Bot. **12**: 577–606. 1925.
9. LUND, E. J.   The polar distribution and significance of continuously maintained electric potentials on the surface of single cells in Pithophora. Abstract. Amer. Soc. Plant Physiol. A.A.A.S. Meeting. December, 1935.
10. MILLER, E. C.   Plant physiology. McGraw-Hill Book Co., Inc. New York. 1938.
11. POPESCO, ST.   Recherches sur la région absorbante de la racine. Buletinul Agriculturii **4**, No. 10–12: 136 pp. 1926.
12. ROSENE, H. F.   Distribution of the velocities of absorption of water in the onion root. Plant Physiol. **12**: 1–19. 1937.
13. ———————.   Comparison of rates of water intake in contiguous regions of intact and isolated roots. Plant Physiol. **16**: 19–38. 1941.
14. STILES, W.   The physiology of plants. p. 51.   John Wiley & Sons. New York. 1938.

15. SIERP, H., and BREWIG, A. Quantitative Untersuchungen über die Wasserabsorptionzone der Wurzeln. Jahrb. wiss. Bot. **82**: 89–122. 1935.

16. THOMÄ, M. über die an gekrümmten Flüssigkeitsoberflächen auftretenden Dampfdruckänderungen. Zeitschr. f. Physik **64**: 224–236. 1930.

17. WILSON, K. The production of root-hairs in relation to the development of the piliferous layer. Ann. Bot. **50**: 121–154. 1936.

EFFECTS OF IRON ON THE GROWTH AND ASH CONSTITUENTS OF *ANANAS COMOSUS* (L.) MERR.[1]

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(WITH ELEVEN FIGURES)

Introduction

Studies on the iron requirements of *Ananas comosus* (L.) Merr. grown under Hawaiian conditions have been made at different times by various investigators (**13, 14**). The consensus of most investigators has been that manganese (**13, 14**) and calcium (**6, 7**) either through the precipitation of water-soluble iron in the soil or in the tissues of the plant were able to cause chlorosis in *A. comosus*. Other conditions, such as different degrees of acidity known either to favor or inhibit the solubility of iron, received at times only little or no attention, probably because methods for measuring H-ion concentrations either electrometrically or colorimetrically had not gained widespread recognition during the first two decades of this century and their application to agricultural problems was at that time in its initial stages.

This paper presents data and a discussion on the effects of "plus-" and "minus-iron" cultures on plant growth and other physiological conditions affecting the values of dry matter, water, ash, electrical resistance, potassium, calcium, magnesium, phosphorus, iron, and manganese in the tissues of *A. comosus* grown in solution cultures supplied either with nitrate or ammonium salts as sources for nitrogen.

The object of growing plants in solution cultures supplied with nitrate or ammonium salts was to study the effects of changes in opposite directions in the H-ion concentration of these solution cultures on the availability of iron to plants. It was observed in former studies that in the course of 2-week intervals H-ion concentrations decreased gradually in nitrate-nitrogen cultures from pH 4.4 to 6.8, and they increased in ammonium-nitrogen cultures from pH 6.6 to 4.2.

Methods

CULTURAL PROCEDURES

Slips (shoots at the apex of vestigial fruits produced laterally on the peduncle) from plants of a single clone of uniform quality and weight were grown for one month in tap water for the formation of roots of approximately uniform length and of equal numbers. They were then transferred to nutrient solution cultures supplied either with nitrate or ammonium salts and other nutrient elements, and either with or without iron, as reported in table I. Each treatment included 16 plants. The various C.P. chemicals employed for the preparation of the nutrient solutions had not been treated

in any way to remove the slight contaminations of iron they contained, and our definition of "minus-iron" cultures is subject to the iron content limitations of C.P. chemicals.

The volume of the containers was approximately 18 liters and the solutions were changed at 2-week intervals. The cultures were constantly aerated and the plants were allowed to grow for about one year; that is, as near as possible to the flowering period. Eight plants from each treatment were then harvested, weighed, and sectioned, following the technique reported in a former publication (23), and analyzed according to methods reported below.

TABLE I

COMPOSITION OF NUTRIENT SOLUTIONS

SALT	MOLAR CONC.	ELE-MENTS	NITRATE SERIES		AMMONIA SERIES	
			− Fe	+ Fe	− Fe	+ Fe
			mg./l.	mg./l.	mg./l.	mg./l.
Ca(NO₃)₂	0.001	N	28.0	28.0	... ...	......
(NH₄)₂SO₄	0.001	N	.	.	28.0	28.0
K₂SO₄	0.0005	K	58.5	58.5	58.5	58.5
KH₂PO₄	0.0005	P	15.5	15.5	15.5	15.5
Ca(NO₃)₂	0.001	Ca	40.0	40.0	. ...	.. ..
CaCl₂	0.001	Ca	....		40.0	40.0
MgSO₄	0.001	Mg	24.0	24.0	24.0	24.0
K₂SO₄, MgSO₄ ...	0.0015	S	48.0	51.0	......	....
K₂SO₄, MgSO₄, (NH₄)₂SO₄ ...	0.0025				80.0	83.0
CaCl₂	0.001	Cl	0.0	0.0	72.0	72.0
FeSO₄	0.0001	Fe	. ...	5.0	.. ..	5.0
Mn, B	....	Micro.	0.5	0.5	0.5	0.5
Zn, Cu, I	....	Micro.	0.1	0.1	0.1	0.1

CHEMICAL PROCEDURES

For an analysis of the ash constituents, 2 grams of oven-dried plant tissues from a composite sample of 8 plants were ashed in a platinum dish. The ash was dissolved in 100 ml. of an aqueous solution of 1.85 per cent. hydrochloric acid. The mixture was then filtered to remove suspended particles of silica and different aliquots of the filtrate were taken for the determination of the various elements mentioned below.

Potassium was determined by the Nitroso-R-salt colorimetric method (18) in a 5-ml. aliquot of the above solution. A 50-ml. aliquot of the solution was used for determining calcium. This was neutralized with 5 N sodium hydroxide using phenolphthalein as indicator, then acidified with 1 ml. of a 50 per cent. solution of acetic acid and subsequently treated with 2 ml. of a 4 per cent. solution of ammonium oxalate. The mixture was allowed to stand overnight, then filtered through asbestos pulp and washed 2 times successively with 20 ml. of water. The precipitate was titrated with 0.05 N potassium permanganate according to the usual procedure and the filtrate was employed for the determination of calcium. An aliquot of the

filtrate from the analysis of calcium, representing 10 ml. of the original volume of the solution, was taken for the determination of magnesium. The 8-hydroxyquinoline colorimetric method (20) was employed for the determination of magnesium. Phosphorus was determined as phosphate-phosphorus in a 2-ml. aliquot of the original solution using the method of BERENBLUM and CHAIN (2) as modified by the senior author (22). The determination of iron was conducted in a 10-ml. aliquot employing the a-a-dipyridyl method of HILL (11) and the orthophenanthroline of SAYWELL and CUNNINGHAM (17). Only the results obtained by the a-a-dipyridyl method are reported here. The determination of iron with a-a-dipyridyl, as conducted by the authors, deviated in certain respects from the original method. The 10-ml. aliquot was neutralized with NaOH using phenolphthalein as indicator and then treated immediately with 1 ml. of a 20 per cent. solution of glacial acetic acid. The ferric iron was reduced with 2 ml. of a 5 per cent. solution of sodium metabisulphite or pyrosulphite ($Na_2S_2O_5$). It was found that most samples of $Na_2S_2O_5$ gave a very slight positive test for Fe^{++} and it was necessary to run a blank at the same time for correction. Manganese was determined in a 10-ml. aliquot using the improved formaldoxime colorimetric method of the senior author (21).

STATISTICAL PROCEDURES

The significance of the difference between the means of the total plant weights of the "plus-iron" and "minus-iron" cultures of the nitrate- and ammonium-nitrogen series was calculated according to FISHER (4, p. 114).

The statistical significance of the differences between the amounts of dry matter, ash, potassium, calcium, magnesium, phosphorus, iron, and manganese in various sections of the plants of the various cultures was calculated from CONRAD's (3) modification of BESSEL's formula.

The significance of the Z values from the above calculations was obtained from Student's table, as calculated by LOVE (15). A summary of the significance of the Z values of the various items is presented in table VIII.

Results

The data concerning plant growth, dry matter, water content, ash and electrical resistance of different sections of the leaves, stem and roots are presented in tables II to V and in figures 1 to 5. Synoptic expressions have been introduced to replace longer ones for certain often repeated appellations. Thus the series nitrate-nitrogen will be designated by N-n and ammonium-nitrogen by A-n. Also the cultures "plus-iron" will be designated by F and "minus-iron" by O.

PLANT WEIGHTS

The data on plant growth in table II and in figure 1 show that the weights of the plants of the F cultures of the N-n series were 33 per cent. greater than those of the plants of the O cultures. The plants of the F and O cultures of the A-n series presented no significant differences in weights.

TABLE II

MEAN, STANDARD DEVIATION, COEFFICIENT OF VARIABILITY, AND PROBABLE ERROR OF THE MEAN OF PLANT AND ROOT WEIGHTS AND OF LENGTHS AND WIDTHS OF THE MATURE (C) AND ACTIVE (D) GROUPS OF LEAVES OF *A. comosus* GROWN IN PLUS- OR MINUS-IRON SOLUTION CULTURES AND SUPPLIED WITH NITRATE SALTS AS SOURCES FOR NITROGEN

| | MINUS IRON | | | | PLUS IRON | | | |
| | | | LEAF GROUPS | | | | LEAF GROUPS | |
	PLANT	ROOTS	MATURE (C) L.* W.*	ACTIVE (D) L. W.	PLANT	ROOTS	MATURE (C) L. W.	ACTIVE (D) L. W.
	gm.	gm.	cm.	cm.	gm.	gm.	cm.	cm.
M	2510	382	64.0 5.8	80.0 6.4	3315	406	77.0 6.2	94.0 6.8
σ	369	61	8.0 0.4	5.0 0.6	455	83	5.0 0.3	10.0 0.4
CV	15	16	12.0 7.0	5.0 9.0	14	20	6.0 5.0	11.0 6.0
PE	78	13	1.7 0.09	1.1 0.13	93	17	1.0 0.06	2.0 0.08
No. of plants	10	10	10	10	11	11	11	11

* L. = length; W. = width.

This suggests that contaminations of iron in the C.P. nutrient salts were sufficient for growth of the plants under the gradually increasing H-ion concentration of the nutrient solutions of the A-n series, which made all traces of iron available to plants. Calculation of the significance of the difference of the means of the total plant weights, according to the method of FISHER as mentioned above, shows that in the N-n series total weights were very significantly greater (100 : 1) in the F cultures.

The weights of roots were not as much affected by either the source of nitrogen or amounts of iron as those of the stem and leaves according to the data in tables II and III and in figure 1, and observed differences lacked significance.

TABLE III

MEAN, STANDARD DEVIATION, COEFFICIENT OF VARIABILITY, AND PROBABLE ERROR OF THE MEAN OF PLANT AND ROOT WEIGHTS AND OF LENGTHS AND WIDTHS OF THE MATURE (C) AND ACTIVE (D) GROUPS OF LEAVES OF *A. comosus* GROWN IN PLUS- OR MINUS-IRON SOLUTION CULTURES AND SUPPLIED WITH AMMONIUM SALTS AS SOURCES FOR NITROGEN

| | MINUS IRON | | | | PLUS IRON | | | |
| | | | LEAF GROUPS | | | | LEAF GROUPS | |
	PLANT	ROOTS	MATURE (C) L.* W.*	ACTIVE (D) L. W.	PLANT	ROOTS	MATURE (C) L. W.	ACTIVE (D) L. W.
	gm.	gm.	cm.	cm.	gm.	gm.	cm.	cm.
M	3176	368	71.0 6.2	86.0 6.7	3151	299	75.0 6.4	83.0 6.6
σ	475	50	6.0 0.6	5.0 0.3	533	64	6.0 0.6	6.0 0.6
CV	15	14	8.0 10.0	6.0 4.0	17	21	8.0 9.0	7.0 9.0
PE	97	10	1.2 0.12	1.0 0.06	128	15	1.4 0.14	1.4 0.14
No. of plants	11	11	11	11	8	8	8	8

* L. = length; W. = width.

DRY MATTER

The weights of dry matter, reported in table IV and in figure 2, differed for comparable sections of the leaves and stem of the plants of the different treatments. The significance of such differences is discussed in greater detail below. The values of dry matter, depicted in figure 2, show that they
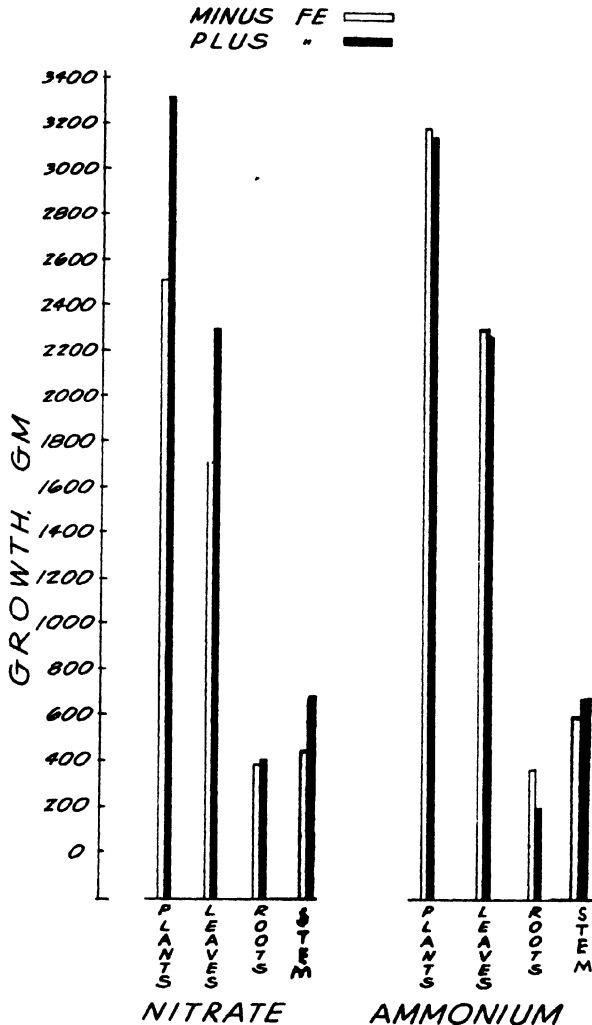
FIG. 1. Growth of pineapple with nitrate and ammonium, with and without iron.

were greater for the F than for the O cultures. Exceptions were the basal (no. 1) and transitional (no. 2) sections of the leaves of the O cultures of the N-n series where the high values of dry matter resulted from decreased tissue succulence through a diminished rate of plant growth. The substances which contributed greatly to the high values of dry matter in plants of the F cultures of both series were sugars, starches, etc., as will be shown in a subsequent publication.
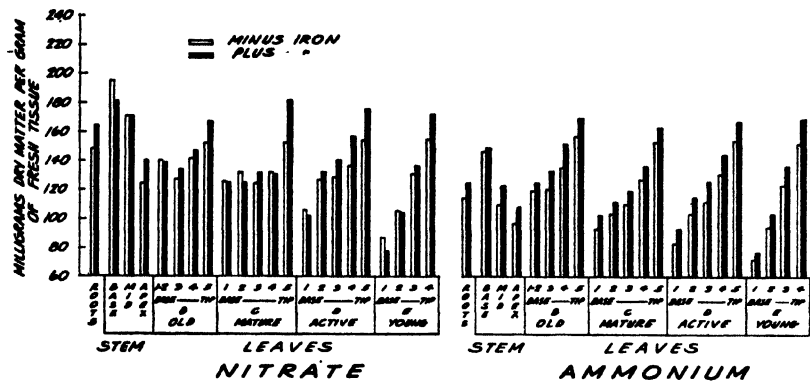
FIG. 2. Dry matter production on nitrate or ammonium, with or without iron.

WATER

The moisture content values of the different sections of the leaves, stem, and roots were inversely proportional to those of dry matter, as shown in table IV and in figure 3. The moisture content was generally higher in

TABLE IV

MILLIGRAMS OF WATER, DRY MATTER, AND ASH PER GRAM OF FRESH TISSUE AND ELECTRICAL
RESISTANCE (OHMS) OF THE EXTRACTED SAP IN DIFFERENT SECTIONS OF THE LEAVES,
STEM AND ROOTS OF ONE-YEAR-OLD PLANTS OF A. comosus GROWN IN PLUS- OR
MINUS-IRON SOLUTION CULTURES AND SUPPLIED WITH NITRATE SALTS
AS SOURCES FOR NITROGEN

PLANT SECTIONS			MINUS IRON				PLUS IRON			
			WATER	DRY MAT-TER	ASH	OHMS	WATER	DRY MAT-TER	ASH	OHMS
Leaves:			mg.	mg.	mg.		mg.	mg.	mg.	
Old (B)	1+2	(base)	860	140	7.70	79.8	861	139	9.32	78.2
	3		873	127	12.57	54.0	866	134	14.60	57.0
	4		859	141	16.90	48.6	853	147	17.05	50.5
	5	(tip)	848	152	18.57	45.6	833	167	17.20	49.8
Mature (C)	1	(base)	875	125	7.12	92.5	878	122	6.10	100.0
	2		868	132	10.18	66.0	877	123	8.86	67.2
	3		876	124	14.90	52.8	869	131	13.36	57.0
	4		868	132	17.95	48.0	869	131	14.92	50.4
	5	(tip)	848	152	20.50	46.8	819	181	18.20	45.0
Active (D)	1	(base)	·894	106	6.15	82.2	898	102	6.38	84.0
	2		874	126	8.06	70.8	871	129	8.12	74.4
	3		872	128	11.34	56.4	869	140	10.93	57.0
	4		864	136	15.76	49.2	844	156	13.90	49.8
	5	(tip)	846	154	19.10	43.2	825	175	14.00	44.4
Young (E)	1	(base)	913	87	7.84	72.6	922	78	7.25	66.0
	2		895	105	7.98	78.0	896	104	7.70	77.4
	3		870	130	10.67	60.0	865	135	9.80	63.0
	4+5	(tip)	846	154	14.50	51.0	829	171	11.28	48.6
Stem:										
Base			805	195	15.60	58.2	819	181	14.10	61.8
Middle			829	171	21.90	48.2	829	171	18.95	48.0
Apex			876	124	16.00	52.2	860	140	15.50	52.2
Roots			852	148	4.73	110.0	836	164	7.38	114.0

TABLE V

MILLIGRAMS OF WATER, DRY MATTER, AND ASH PER GRAM OF FRESH TISSUE AND ELECTRICAL
RESISTANCE (OHMS) OF THE EXTRACTED SAP IN DIFFERENT SECTIONS OF THE LEAVES,
STEM AND ROOTS OF ONE-YEAR-OLD PLANTS OF *A. comosus* GROWN IN PLUS- OR
MINUS-IRON SOLUTION CULTURES AND SUPPLIED WITH AMMONIUM
SALTS AS SOURCES FOR NITROGEN

PLANT SECTIONS			MINUS IRON				PLUS IRON			
			WATER	DRY MATTER	ASH	OHMS	WATER	DRY MATTER	ASH	OHMS
			mg.	*mg.*	*mg.*		*mg.*	*mg.*	*mg.*	
Leaves:										
Old (B)	1+2 (base)		881	119	7.55	93.0	876	124	7.44	82.2
	3		880	120	11.66	61.8	868	132	12.94	57.6
	4		865	135	11.34	57.6	849	151	12.55	54.6
	5	(tip)	844	156	10.92	55.2	831	168	12.00	51.6
Mature (C)	1	(base)	907	93	5.93	97.8	898	102	6.23	87.6
	2		897	103	9.58	68.4	889	111	9.10	62.4
	3		890	110	11.00	61.2	881	119	11.11	55.2
	4		874	126	12.36	55.8	864	136	13.06	51.6
	5	(tip)	848	152	11.10	54.6	838	162	14.30	52.2
Active (D)	1	(base)	917	83	6.16	93.0	907	93	6.13	84.0
	2		897	103	7.00	73.8	886	114	7.18	69.6
	3		889	111	10.54	56.4	875	125	11.76	52.2
	4		870	130	12.50	51.6	856	144	13.52	49.8
	5	(tip)	847	153	10.25	51.0	834	166	15.93	48.6
Young (E)	1	(base)	928	72	6.40	82.2	923	77	6.78	73.8
	2		906	94	7.23	78.0	897	103	5.77	74.4
	3		878	122	9.27	60.0	865	135	9.73	62.4
	4+5 (tip)		849	151	10.12	51.6	832	168	8.56	52.2
Stem:										
Base	.. ..		854	146	11.23	72.6	850	150	9.95	63.0
Middle	.. ..		891	109	16.10	52.2	878	122	13.90	51.0
Apex	.. . .		903	97	12.12	56.4	892	108	11.88	55.2
Roots	....		886	114	2.39	115.0	876	124	5.70	85.8
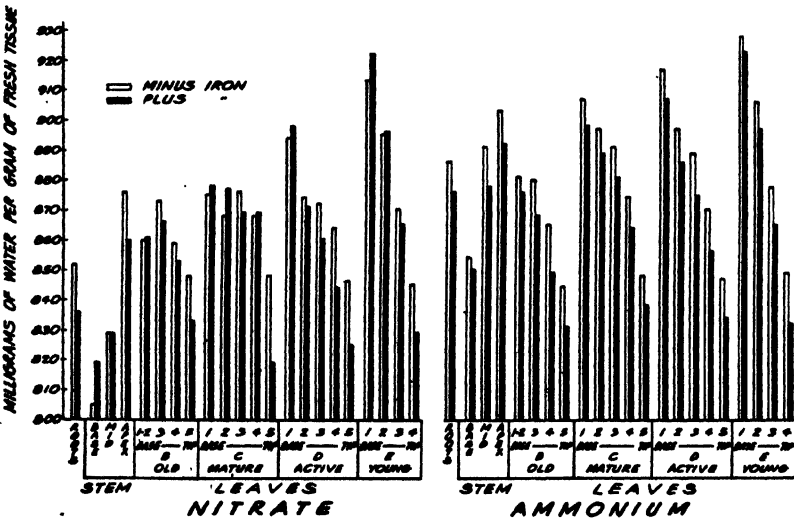
FIG. 3. Water content of tissues grown with nitrate or ammonium, and with or without iron.

sections containing meristematic tissues than in tissues of advanced chrono-
logical and physiological age. Moisture content values were greater in most
plant sections from the O than F cultures of both series. Exceptions were
the basal sections of leaves and apical ones of the stem of the N-n series.

The moisture content was generally higher for the plants of the A-n than
of the N-n series, a phenomenon also observed in former studies (23).

ASH

The ash values, reported in table V and in figure 4, were generally higher
for the plants of the N-n than of the A-n series. Differences between the
ash content of the F and O cultures were not generally consistent. Many
of the leaf and stem sections from the O cultures of the N-n series contained
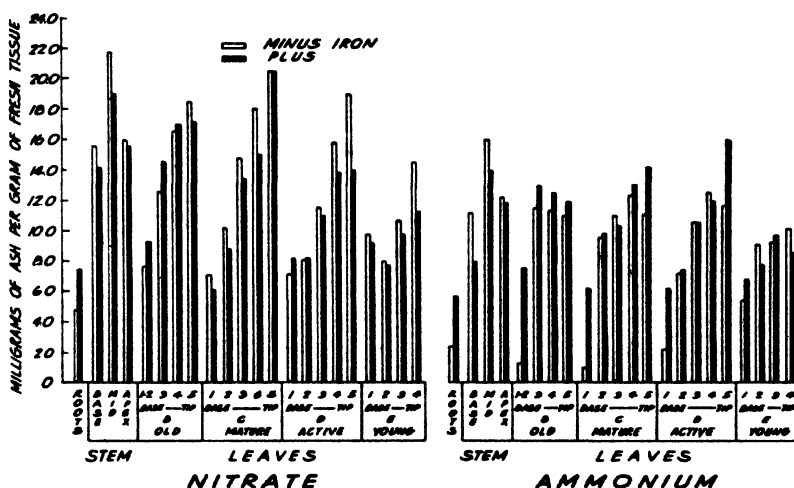
FIG. 4. Ash content of tissues from plants grown on nitrate or ammonium, with or
without iron.

more ash than comparable sections from the F cultures of the same series.
This might have resulted from so-called concentration effects, that is, from
salt accumulations caused by differences in the weights of the plants of the
different treatments rather than from differences in the rates of ash con-
stituents entering their tissues. The results obtained from the A-n series
indicate that slightly greater concentrations of ash-forming elements had
accumulated in the tissues of most sections of the plants of the F than of
the O cultures. Evidence (below) suggests that ammonium ions (NH_4^+)
decrease or retard the absorption of other cations, especially of calcium,
which might account for the generally lower values of ash in the plants of
the A-n series.

ELECTRICAL RESISTANCE

The values of the relative electrical resistance (ohms) of the extracted
sap of the various sections, reported in tables IV and V and in figure 5,
were higher, with a few exceptions, in the sections of the plants of the F

than O cultures of the N-n series. This relationship was reversed in the A-n series. Electrical resistance values were approximately inversely proportional to ash values for comparable plant sections, as shown in tables IV and V. Owing to the fact that such values may be increased by weak electrolytes such as carboxylic acids or amino acids which occur in various amounts in different plant sections, or decreased by non-electrolytes such as sugars through interference of ionic movements, they are of limited value for predicting total ash values because they represent the sum total of the conductance of all inorganic and organic electrolytes and non-electrolytes.

POTASSIUM

The data reported in tables VI and VII and in figure 6 show that the plants of the O cultures of the N-n series contained, with few minor excep-
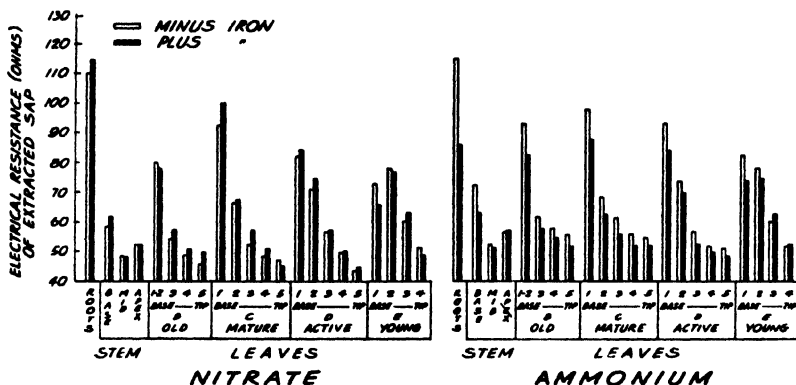
FIG. 5. Electrical resistance of sap from plants grown on nitrate or ammonium, with or without iron.

tions, slightly higher amounts of potassium than those of the F cultures of the same series. Moreover, they suggest that the rate of potassium absorption by the plants of the O cultures of the N-n series remained constant, while that of growth was appreciably reduced by the lack of adequate amounts of iron, resulting in a greater concentration of potassium per plant volume. The reverse of this condition occurred, with the exception of a few terminal (no. 5) leaf sections, in the plants of the O cultures of the A-n series which contained slightly smaller amounts of potassium than those of the F cultures. Comparing the amounts of potassium in the plants of the F cultures between the N-n and A-n series, we observe that they were slightly greater in many sections of the plants of the latter than former series. The concentration gradient of potassium between the basal and the terminal sections of the leaves varied from 200 to 400 per cent. in different groups of leaves, whereas in the stem it varied relatively little.

CALCIUM

The differences in the amounts of calcium between the plants of the O and F cultures of the same nitrogen series were relatively small as reported

TABLE VI

MILLIGRAMS OF POTASSIUM, CALCIUM, MAGNESIUM, AND PHOSPHORUS, AND MICROGRAMS OF IRON AND MANGANESE PER GRAM OF FRESH TISSUE IN DIFFERENT SECTIONS OF THE LEAVES, STEM AND ROOTS OF ONE-YEAR-OLD PLANTS OF *A. comosus* GROWN IN PLUS- OR MINUS-IRON SOLUTION CULTURES AND SUPPLIED WITH NITRATE SALTS AS SOURCES FOR NITROGEN

PLANT SECTIONS		MINUS IRON						PLUS IRON					
		K	Ca	Mg	P	Fe	Mn	K	Ca	Mg	P	Fe	Mn
		mg.	mg.	mg.	mg.	γ	γ	mg.	mg.	mg.	mg.	γ	γ
Leaves:													
Old (B)	1 + 2 (base)	5.84	0.26	0.14	0.20	0.22	0.45	6.43	0.30	0.10	0.09	0.43	0.45
	3	8.44	0.51	0.38	0.26	0.18	0.37	8.75	0.64	0.46	0.13	0.50	0.35
	4	9.38	0.89	0.62	0.28	0.20	0.79	9.18	0.93	0.66	0.17	0.61	0.80
	5 (tip)	11.85	0.90	1.12	0.29	0.35	1.41	8.82	1.31	0.97	0.22	0.77	0.95
Mature (C)	1 (base)	4.47	0.33	0.17	0.06	0.18	0.18	3.82	0.46	0.11	0.08	0.44	0.37
	2	8.25	0.27	0.11	0.13	0.15	0.58	8.06	0.31	0.06	0.13	0.33	0.30
	3	10.37	0.52	0.31	0.17	0.16	0.87	8.95	0.50	0.23	0.14	0.38	0.29
	4	12.00	0.78	0.62	0.21	0.22	1.18	8.95	0.63	0.39	0.14	0.59	0.43
	5 (tip)	14.60	0.78	0.76	0.25	0.20	2.43	12.12	1.09	0.65	0.22	0.86	0.94
Active (D)	1 (base)	3.49	0.26	0.16	0.05	0.12	0.17	3.82	0.43	0.20	0.07	0.34	0.16
	2	5.24	0.21	0.12	0.08	0.14	0.25	5.97	0.36	0.11	0.11	0.41	0.21
	3	8.00	0.31	0.25	0.11	0.14	0.36	7.62	0.45	0.19	0.11	0.44	0.22
	4	11.34	0.41	0.39	0.14	0.15	0.82	8.62	0.54	0.29	0.11	0.49	0.41
	5 (tip)	13.32	0.67	0.54	0.21	0.20	1.66	9.67	0.67	0.44	0.13	0.50	0.69
Young (E)	1 (base)	4.57	0.20	0.33	0.05	0.17	0.16	4.88	0.26	0.27	0.07	0.28	0.27
	2	5.14	0.18	0.21	0.06	0.16	0.27	4.88	0.29	0.21	0.08	0.31	0.25
	3	6.92	0.23	0.25	0.10	0.22	0.31	6.40	0.36	0.22	0.09	0.35	0.38
	4 + 5 (tip)	9.62	0.42	0.45	0.14	0.32	0.99	8.10	0.59	0.50	0.10	0.48	0.64
Stem:													
Base		4.76	2.65	0.67	0.07	0.35	0.43	5.55	2.46	0.60	0.09	0.52	0.47
Middle		7.12	3.42	0.75	0.20	0.22	0.44	6.41	3.42	0.43	0.12	0.46	0.41
Apex		7.32	1.71	0.67	0.18	0.20	0.32	7.00	2.24	0.44	0.14	0.40	0.31
Roots		2.37	0.24	0.22	0.05	1.17	0.38	1.64	0.45	0.32	0.08	21.90	0.61
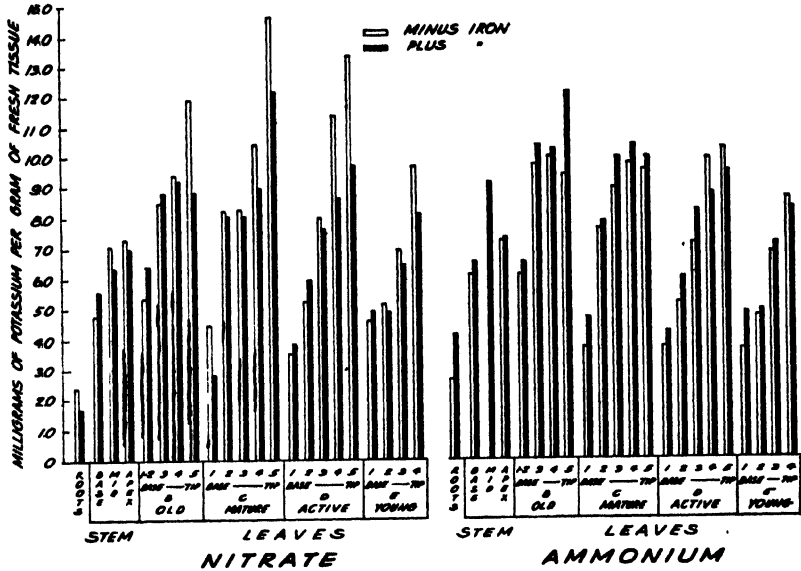
FIG. 6. Potassium content of tissues from plants grown with nitrate or ammonium, with or without iron.

in tables VI and VII and in figure 7. However, those between different nitrogen series, namely between the A-n and N-n series but of comparable iron cultures, were relatively great. The calcium content of the stem in the
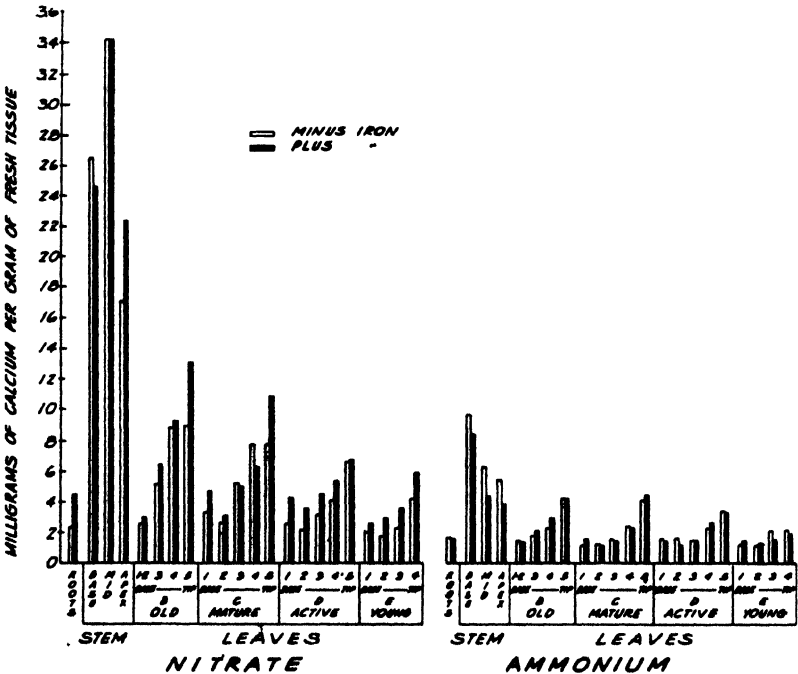
FIG. 7. Calcium content of tissues from plants on nitrate or ammonium, with or without iron.

TABLE VII

MILLIGRAMS OF POTASSIUM, CALCIUM, MAGNESIUM, AND PHOSPHORUS AND MICROGRAMS OF IRON AND MANGANESE PER GRAM OF FRESH TISSUE IN DIFFERENT SECTIONS OF THE LEAVES, STEM, AND ROOTS OF ONE-YEAR-OLD PLANTS OF *A. comosus* GROWN IN PLUS- OR MINUS-IRON SOLUTION CULTURES AND SUPPLIED WITH AMMONIUM SALTS AS SOURCES FOR NITROGEN

PLANT SECTIONS		MINUS IRON						PLUS IRON					
		K	Ca	Mg	P	Fe	Mn	K	Ca	Mg	P	Fe	Mn
		mg.	*mg.*	*mg.*	*mg.*	γ	γ	*mg.*	*mg.*	*mg.*	*mg.*	γ	γ
Leaves:													
Old (B)	1 + 2 (base)	6.07	0.15	0.31	0.15	0.24	0.30	6.52	0.15		0.14	0.37	0.12
	3	9.67	0.18	0.36	0.19	0.27	0.63	10.30	0.21	0.22	0.37	1.20	0.33
	4	9.94	0.23	0.81	0.30	0.38	0.64	10.20	0.29		0.35	1.60	0.66
	5 (tip)	9.33	0.42	0.90	0.37	0.52	2.16	12.08	0.42	0.44	0.42	1.49	1.08
Mature (C)	1 (base)	3.72	0.12	0.67	0.08	0.22	0.16	4.64	0.16	0.17	0.10	0.42	0.10
	2	7.58	0.13	0.32	0.19	0.23	0.16	7.83	0.12		0.09	0.47	0.17
	3	8.87	0.16	0.41	0.23	0.29	0.30	9.93	0.15	0.14	0.19	0.62	0.24
	4	9.68	0.24	1.18	0.26	0.32	0.41	10.30	0.22	0.17	0.24	1.02	0.51
	5 (tip)	9.50	0.41	1.08	0.36	0.35	1.41	9.90	0.45	0.59	0.30	1.66	0.93
Active (D)	1 (base)	3.70	0.16	0.40	0.07	0.21	0.10	4.23	0.15	0.15	0.07	0.34	0.09
	2	5.15	0.17	0.29	0.10	0.23	0.13	6.00	0.12	0.08	0.08	0.46	0.12
	3	7.12	0.15	0.38	0.15	0.25	0.11	8.18	0.15	0.16	0.08	0.50	0.16
	4	9.86	0.23	0.54	0.22	0.24	0.20	8.68	0.27	0.25	0.16	0.66	0.29
	5 (tip)	10.20	0.34	0.77	0.23	0.38	0.50	9.43	0.34	0.25	0.17	1.08	0.63
Young (E)	1 (base)	3.60	0.13	0.40	0.06	0.18	0.13	4.81	0.15	0.29	0.05	0.33	0.08
	2	4.70	0.12	0.28	0.07	0.22	0.16	4.90	0.13	0.19	0.06	0.40	0.13
	3	6.78	0.21	0.61	0.11	0.26	0.24	7.10	0.16	0.25	0.08	0.57	0.20
	4 + 5 (tip)	8.57	0.22	0.61	0.11	0.35	0.32	8.25	0.19	0.33	0.09	0.67	0.42
Stem:													
Base		6.08	0.97	1.08	0.13	0.31	0.15	6.52	0.85	0.31	0.14	0.35	0.19
Middle		9.00	0.64	1.82	0.20	0.23	0.16	9.10	0.44	0.94	0.14	0.43	0.12
Apex		7.22	0.54	2.02	0.14	0.24	0.13	7.30	0.39	0.98	0.12	0.35	0.11
Roots		2.66	0.16	0.45	0.04	0.33	0.29	4.13	0.16	0.32	0.07	28.20	0.50

N-n series was from 2.5 to 5.5 times greater than that in the A-n series. The leaves of the F cultures contained more calcium than those of the O cultures in the N-n series. The results generally emphasize the fact that the absorption of calcium by *A. comosus* was greatly accelerated by nitrate ions and retarded by ammonium ions, while the extra amounts of iron in the F cultures increased calcium absorption only slightly over those of the O cultures. The data suggest that the acceleration of calcium absorption by nitrate ions and its retardation by ammonium ions could have been caused by electrostatic forces as postulated in the theory of GIRARD (7) and HAYNES (10). In conformity, then, with the above theory of absorption the NO_3^-

TABLE VIII

STATISTICAL SIGNIFICANCE OF THE DIFFERENCE* BETWEEN CONSTITUENTS OF COMPARABLE SECTIONS OF PLANTS GROWN IN PLUS- AND MINUS-IRON CULTURES SUPPLIED EITHER WITH NITRATE OR AMMONIUM SALTS AS SOURCES FOR NITROGEN†

| | NITRATE-N | | | | AMMONIUM-N | | | |
| | LEAVES | | STEM | | LEAVES | | STEM | |
CONSTIT-UENTS	APPROX-IMATE SIGNIFI-CANCE	IN FAVOR OF CUL-TURE	SIG-NIFI-CANCE	IN FAVOR OF CUL-TURE	SIG-NIFI-CANCE	IN FAVOR OF CUL-TURE	SIG-NIFI-CANCE	IN FAVOR OF CUL-TURE
Dry matter	300 : 1	+ Fe	None		9999 : 1	+ Fe	25 : 1	+ Fe
Ash	500 : 1	– Fe	None		40 : 1	+ Fe	None	
Potassium	600 : 1	– Fe	None		180 : 1	+ Fe	40 : 1	– Fe
Calcium	9999 : 1	+ Fe	None		None		85 : 1	– Fe
Magnesium	2000 : 1	– Fe	19 : 1	– Fe	9999 : 1	– Fe	260 : 1	– Fe
Phosphorus	250 : 1	– Fe	None		None		None	
Iron	9999 : 1	+ Fe	25 : 1	+ Fe	9999 : 1	+ Fe	None	
Manganese	250 : 1	– Fe	None		None		None	

* Calculated by CONRAD's formula and from LOVE's table.
† The significance of the difference (represented by the algebraic sum of the differences of items between comparable plant sections from different treatments) is not used in the strict sense of statistics but as a synoptic index of all such differences.

in the N-n series was electrostatically attracted by Ca^{++} and possibly by other cations, while that of Ca^{++} in the A-n series was electrostatically repelled by NH_4^+ and possibly other cations. It is difficult, however, to explain on the basis of the effects of NO_3^- and NH_4^+ on calcium absorption, why the absorption of potassium and, to a certain degree, of magnesium by roots was not affected in a similar manner in conformity with the above mentioned electrostatic theory. Calcium accumulated in the stem in very great proportions with most of it located in the tissues of the stele (24). This element did not move as freely as potassium from the tissues of the stem to the leaves, as indicated by data showing that the stem contained from 2 to 5 times more calcium than the leaves, while potassium accumulated mostly in the terminal sections of the latter organs. The causes for the low rates of calcium translocation from the stem to the leaves are possibly

anatomical and compare well with rates usually attributed to diffusion. Moreover, the accumulation of calcium in the apical tissues of the stem before the flowering period and its depletion thereafter indicate an exceedingly slow movement of this element through the meristematic tissues of the stem apex during the above period which, however, changes very rapidly as soon as the meristematic tissues of the apical region of the stem undergo differentiation during the period of fructification. This postulation has been supported by some anatomical evidence which shows that the apical tissues of the stem and those of the leaf bases lack, during their meristematic stages, well defined and adequately functioning tracheids. In regions of the stem which have advanced in development beyond the meristematic stage, as in the medial region, the conduction of substances from
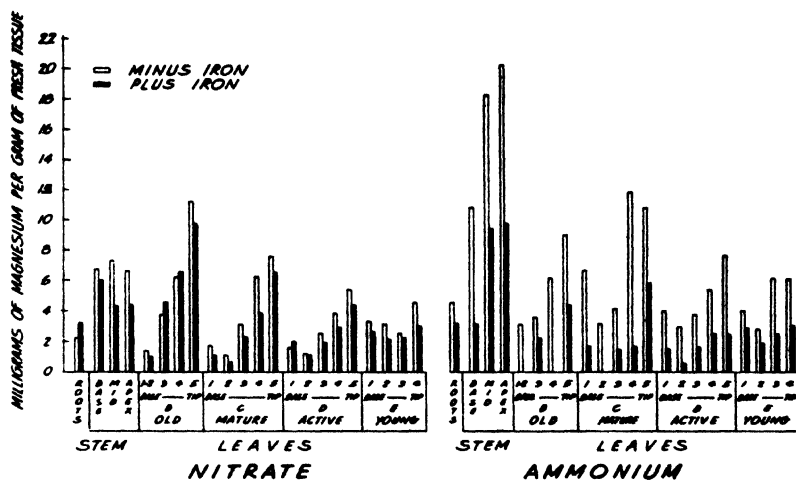
FIG. 8. Magnesium content of tissues from plants grown with nitrate or ammonium, with or without iron.

the tissues of the stem to those of the leaves is more rapid than in the apical region before the flowering period, suggesting that the movement of substances is not dependent on diffusion forces but that it is carried on through well-formed tracheids. The amount of anastomosis between the tracheids of the stem and those of the basal sections of the leaves has not been definitely ascertained.

MAGNESIUM

The distribution of magnesium in different sections of the plants of the various cultures, reported in tables VI and VII and in figure 8, shows that the plants of the O cultures of both nitrogen series contained, with few exceptions, greater quantities of this element than the plants of the F cultures. The plants of the A-n series of the O cultures contained greater amounts of magnesium than those of the corresponding cultures of the N-n series. In the F cultures the average values of magnesium were greater in the leaves of the plants of the N-n than of the A-n series, but in the stem

they were reversed. Magnesium accumulated, although not to the same extent, in the stem of the plants of all treatments. The much greater accumulations of magnesium in the stem of the plants of the O cultures of the A-n series than in any of the other cultures cannot be attributed to concentration effects resulting from differences in plant weights because the plants of the A-n series weighed approximately the same or more than those of the N-n series of comparable iron treatments.

<center>PHOSPHORUS</center>

The distribution of phosphorus in the plants of the various cultures, reported in tables VI and VII and in figure 9, shows that it accumulated in greater amounts in the leaves of the old (B) and mature (C) groups of the plants of the A-n than of the N-n series. The differences between the amounts of phosphorus in the leaves of the active (D) and young (E)
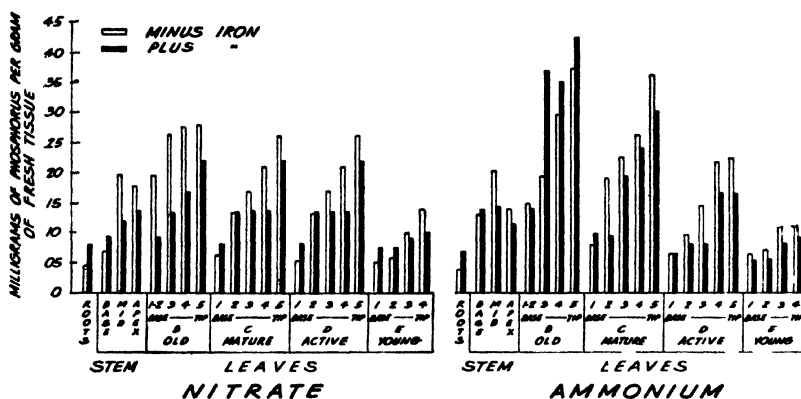
FIG. 9. Phosphorus content of tissues from plants grown on nitrate or ammonium, with or without iron.

groups of the plants of the two nitrogen series were small and irregularly distributed. Many of the sections of the plants of the O cultures of both nitrogen series contained appreciably greater amounts of phosphorus than comparable sections of the plants of the F cultures.

The amounts of phosphorus in roots, although generally very small, were higher for those in the F than in the O cultures of both nitrogen series. It is possible that the slightly greater amounts of phosphorus in the roots of the F than O cultures resulted from the precipitation of some phosphate-phosphorus by iron on the surface. A similar possibility is also suggested by the analytical data on iron.

<center>IRON</center>

The distribution of total iron in the plants of the different treatments and especially in those of the O cultures is very interesting. It was mentioned earlier that iron, in concentrations of 5 mg. per liter of nutrient solution, was added only to the F but not to the O cultures.

The data in tables VI and VII and in figure 10 show that the amounts of iron were generally higher in the plants of the F than of the O cultures. They also show that the plants of the A-n series contained more iron than those of the N-n series. The highest values of iron were found in the roots of the plants of the F cultures of both nitrogen series. The roots of the N-n series of the O cultures contained more iron than those of the corresponding iron treatment of the A-n series. The causes for the accumulation of greater amounts of iron in the stem and leaf tissues of the plants of the A-n than N-n series could be attributed to differences in the availability of iron caused by changes in the H-ion concentration of the solution cultures of the two series. The H-ion concentrations of the cultures of the A-n series (supplied with ammonium sulphate) changed in the course of
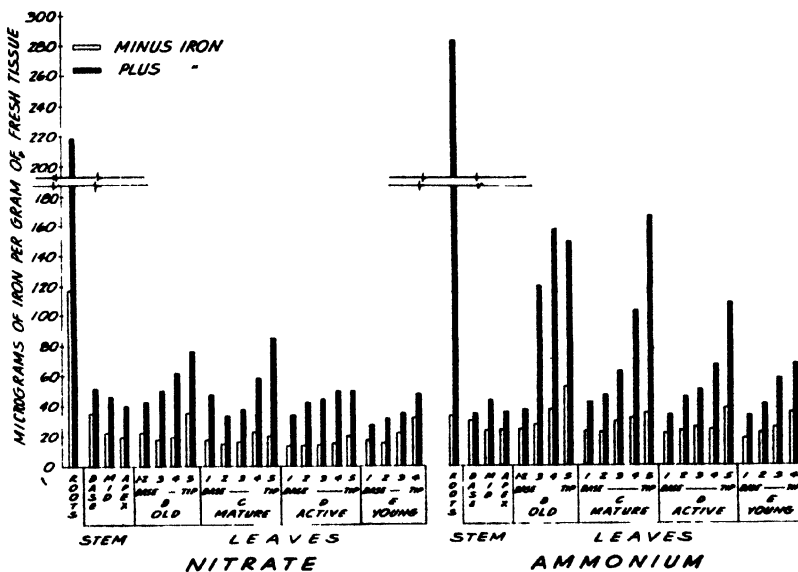
FIG. 10. Iron content of tissues from plants grown on nitrate or ammonium, with or without iron.

two weeks from an initial value of pH 6.6 to a final one of 4.2, and in those of the N-n series (supplied with potassium or calcium nitrate or with both) from pH 4.4 to 6.8. The changes in the initial H-ion concentrations were caused, in the case of the cultures of the A-n series, by a greater rate of absorption of ammonium than of sulphate ions and in that of the N-n series by a greater one of nitrate than of calcium and possibly other cations. The H-ion concentration changes from pH 6.4 to 5.0 in the cultures of the A-n series were relatively rapid, effected mostly in the course of 3 days, and pH values ranging between 4.0 and 5.0 were maintained for the greater portion of the two-week period which favored iron solubility. The results suggest that a great portion of the water-soluble iron of the cultures of the N-n series was possibly precipitated from the solution during the changes in the H-ion concentration from pH 4.4 to 6.4, which condition decreased also the availability of this element to the plants.

The relatively great amounts of iron found in the roots of the plants of the F cultures, as reported in tables VI and VII, resulted, in the opinion of the writers, from iron which was deposited on the surface of the roots during the processes of absorption. Partial confirmation of the precipitation of iron on the superficial tissues of the roots was obtained by suspending well-washed, intact roots in a 2 per cent. solution of sodium hydrosulphite for 4 hours and then analyzing the solution for iron. The availability of iron precipitated on the surface of the roots at favorable H-ion concentrations can be demonstrated if chlorotic plants of *A. comosus* containing such iron on their roots are placed in solution cultures supplied with ammonium sulphate instead of calcium nitrate, or if the pH of the solution is maintained at constant pH values of 4.0 to 5.0. It is also possible that some iron was precipitated in nutrient solutions of the N-n series during the generation of high pH values by the absorption of greater amounts of anions than cations. The greater amounts of iron in the roots of the plants of the F cultures of the A-n than of the N-n series may be explained by the postulation that more iron came in contact with the roots of these plants because of more favorable H-ion concentrations for the solubility of iron in the A-n series. In the case of the roots of the plants of the O cultures, however, the amounts of iron precipitated on the roots of the plants of the A-n series were lower than those of the N-n series because of the highly restricted amounts of iron in the cultures of both series. Also, the higher H-ion concentrations of the cultures of the A-n than of the N-n series, and the scarcity of iron in the O cultures prevented the precipitation and possible deposition of this element on the surface of the roots of the plants of the A-n series, whereas some precipitation of iron was effected by the high pH values of the cultures on the roots of the plants of the N-n series. However, the precipitation of iron on the roots of the plants of the O cultures of the N-n series represented by the differences between the amounts of iron in the O cultures of the A-n and N-n series $(1.17 - 0.33 = 0.84)$ demonstrates the efficacy of high pH values on iron precipitation even in solution cultures where the concentrations of this element could not have been any higher than 10^{-8} gm. per liter of solution. Therefore, the absorption of iron by roots may be conditioned in part either by the initial acidity of the nutrient solution or by such subsequent changes in the acidity that develop on the surface of the roots during the absorption of nutrient elements. The precipitation of iron on the surface of the roots of the plants of the O cultures of the N-n series is a paradoxical phenomenon because the quantities of this element, occurring as mere contaminations in the C.P. nutrient salts, were exceedingly small. The precipitation of this element under the conditions of the N-n cultures, however, indicates its great susceptibility to insolubleness and hence its low rate of permeability into the tissues of the roots. The possible precipitation of some iron by phosphorus has been suggested by the results of the chemical analyses.

MANGANESE

The distribution of manganese in the plants of the various treatments is reported in tables VI and VII and in figure 11. The data show that manganese occurred, in the majority of cases, in much greater amounts in the plants of the N-n than of the A-n series. It was generally higher in the stem and leaves but not in the roots of the plants of the O cultures of the N-n series. The smaller amounts of manganese in the leaves and stem of the plants of the F than of the O cultures of the N-n series may be attributed to iron precipitated on the surface of the roots which presumably reduced the rate of manganese permeability into the root tissues. With the amounts of both iron and manganese being greater in the roots of the plants of the
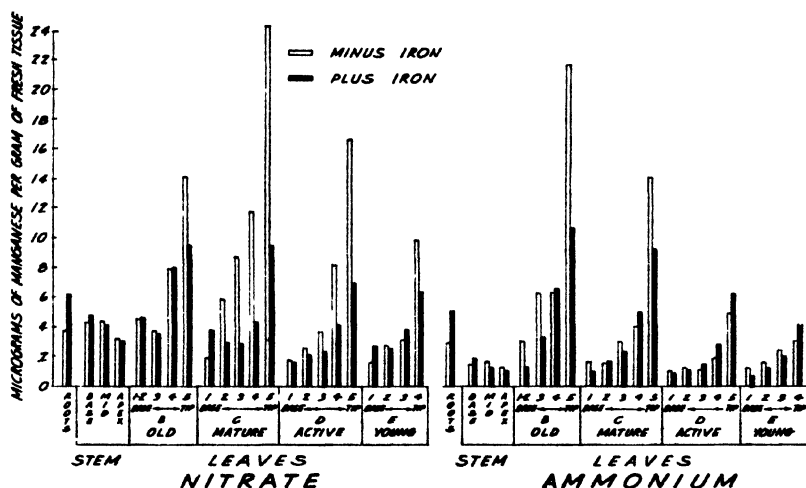
FIG. 11. Manganese content of tissues from plants grown on nitrate or ammonium, with or without iron.

F than of the O cultures, while those of the latter element were reversed in the sections of the leaves and stem, the data suggest that manganese was probably precipitated, like iron, on the surface of the roots.

Analyses of the data in tables VI and VII show that the effects of different pH reactions, as mentioned above for nutrient cultures supplied either with ammonium sulphate or calcium nitrate salts, caused depositions of manganese on the roots of the plants of the O cultures of 0.38 and 0.29 microgram of Mn per gram of fresh tissue for the N-n and A-n series, respectively, equivalent to 23.7 per cent. $\left(\dfrac{0.38 - 0.29}{0.38} \times 100\right)$ in favor of the N-n series. The amounts of manganese depositions in the roots of the plants of the F cultures were 0.61 and 0.50 microgram per gram of fresh tissue for for the N-n and A-n series, respectively, equivalent to 18.0 per cent. $\left(\dfrac{0.61 - 0.50}{0.61} \times 100\right)$ in favor of the N-n series. The effects of iron on the deposition of Mn in the roots of the plants of the A-n series were 0.50 and

0.29 microgram per gram of fresh tissue for the F and O cultures, respectively, equivalent to 42.0 per cent. $\left(\dfrac{0.50 - 0.29}{0.50} \times 100\right)$ in favor of the F cultures. In the plants of the N-n series the Mn depositions in the roots were 0.61 and 0.38 microgram per gram of fresh tissue for the F and O cultures, respectively, equivalent to 46 per cent. $\left(\dfrac{0.61 - 0.38}{0.61} \times 100\right)$ in favor of the F cultures. Therefore, the precipitation of Mn caused by the iron content of the F *vs.* the O cultures was greater $(42 + 46 \div 2 = 44$ per cent.) than that by the different pH values of the cultures of the N-n *vs.* A-n series $(23.7 + 18.0 \div 2 = 20.8$ per cent.).

The data demonstrate also that the relative (low) acidity of the nutrient solutions had affected only the precipitation of manganese on the roots but it did not interfere with its movement into the tissues of the stem and leaves. The relatively higher values of manganese in the plants of the O cultures of the N-n series, however, might have resulted from concentration effects on account of the smaller weights of the former plants.

Discussion

The results presented above indicate that the changes in H-ion concentrations which constantly develop on the surface of roots or at the regions of the rhizosphere during the absorption of ions from nutrient solution cultures supplied either with nitrate or with ammonium salts as sources for nitrogen may facilitate or inhibit the absorption of iron by roots. The H-ion concentration changes resulting from such conditions may vary both in magnitude and in duration as they depend on the chemical composition of the nutrient solution and on the length of the period of absorption of certain ions by roots. In view of the fact that iron solubility in nutrient solutions and possibly in soils, and its availability to *A. comosus* depends on a favorable range of hydrogen ion concentrations of the nutrient solution and of soils, an evaluation of both pH and water-soluble iron constitutes the only reliable criterion for inorganic iron availability. The generation of acidic and basic reactions at the rhizosphere through the absorption by plant roots of anions and cations at different rates influences the solubility of iron at the point of its entrance into the roots. Any conditions interfering with the solubility of iron in the solution culture or soil render meaningless any comparisons between the initial amounts of iron in the nutrient solution and those absorbed by plants.

The amounts of iron in the basal (1) sections of leaves (the first point of entrance of this element into the leaves from the stem) of the O cultures were slightly smaller in the N-n than A-n series. In spite of the lack of appreciable differences in the concentrations of iron in the basal (1) sections of the young leaves (E) of the O cultures between the N-n and A-n series the difference in chlorophyll between the two series were very great as will be discussed further in a subsequent publication. In the remaining sections

of all leaves of the O and F cultures the amounts of iron were generally higher in the plants of the A-n than N-n series. This indicates that the movement of iron into the leaves continued probably for a longer period and was possibly of a greater intensity in the A-n than N-n series. These findings conform with the postulation advanced above that the quantities of available iron decrease rapidly in solution cultures supplied with nitrate salts (calcium or potassium nitrate) because of the generation of H-ion concentrations (high pH) incompatible with the solubility of iron.

OLSEN (16) has suggested the possibility of iron precipitation as iron phosphate within the water-conducting vessels of *Zea mays* L. plants, and WADLEIGH *et al.* (26) have extended OLSEN's hypothesis to include the effect of the pH of the sap on iron solubility. The presence of oxidizing agents for the precipitation of iron in plant tissues has also been cited by others (6). In order to obtain some idea of the influence of the relative acidity and reducing power of the sap on iron solubility, determinations of both titratable acidity and H-ion concentration and of ascorbic acid were made. These will be reported in a subsequent publication. They suggest, however, that iron in the leaves is susceptible neither to precipitation nor to oxidation from a lack of sufficient acidity or of reducing substances.

The data on the distribution of iron in the sections of the plants of the O cultures of the A-n series, which produced weights almost as great as those of the F cultures, suggest that the absolute iron requirements of *A. comosus* under highly favorable conditions for iron solubility are relatively small (approximately 10^{-7} mg. per liter of solution). They also demonstrate that plant growth, although favored more by the addition of somewhat greater amounts of iron than the mere traces occurring in the O cultures derived from impurities in the C.P. salts, is not affected by excessive amounts, and that such amounts are of no additional value to plants except in nutrient solutions which are subject to pH changes from low to high levels as in those supplied with nitrate salts. Definite limits for iron requirements cannot be set on the basis of plant tissue analyses because the iron content of such tissues is influenced by concentration or dilution effects caused by various complex factors, as plant growth rate and age, besides the readily available iron content of the substratum.

Our knowledge of the physiological functions of iron in plants is limited except for its catalytic properties in the formation of chlorophyll, or more probably of protochlorophyll, and its participation in the composition of catalase and certain cytochromes (1). The facts obtained so far suggest that, as long as plants are supplied with iron in concentrations sufficient to catalyze the synthesis of the required amounts of chlorophyll, any greater quantities that may be absorbed by the roots are possibly of no additional physiological value. The fact that greater amounts of iron are often found in the tissues of chlorotic than of green plants constitutes an anomaly and a paradox. It is assumed that such accumulations of iron in the tissues of chlorotic plants result from amounts translocated there after such tissues

ceased to grow and a great deal of their initial metabolic activity and possibly the power of forming more chlorophyll has been appreciably reduced. In all such chlorotic plants the new or meristematic tissues rather than the chronologically and physiologically more advanced tissues of the plants contain less iron than those of non-chlorotic or green plants. Amounts of chlorophyll rather than of iron in plant tissues should be the criteria for evaluating plant response to iron treatments. As the quantities of chlorophyll are also influenced by those of available nitrogen, any deficiencies of the latter are likely to reduce considerably the amounts of chlorophyll in the leaves. Reductions of chlorophyll amounts below certain minimum values directly affect the carbohydrate-synthesizing efficiency of the plant. Thus any deficiencies of iron or nitrogen causing reductions in chlorophyll values influence the rate of plant growth, carbohydrate synthesis, assimilation, nutrient element absorption, and accumulation. It is possible that all such conditions had developed in the plants of the O cultures of the N-n series and caused the production of small plant weights, some accumulations of iron and other elements, and various other physiological disturbances.

The data on the distribution of ash show that the plants of the O cultures of the N-n series contained more of these substances than those of the other cultures because of concentration effects resulting from the smaller weights of the plants of the O cultures. The amounts of ash in the leaves of the F cultures of the A-n series were greater than those of the O cultures, suggesting a greater rate of absorption of ash constituents by the plants of the F than of the O cultures. The amounts of ash in the stem of the O cultures were reversed, suggesting a reduced rate of translocation. In view of the fact that the weights of all the plants of the A-n series were about the same, the greater amounts of ash constituents in the leaves of the F than of the O cultures should be attributed to their greater content of chlorophyll and its effects on the translocation of solutes and normal physiological functioning of the plants.

The distribution of phosphorus shows that slightly greater amounts of this element were deposited in the roots of the plants of the F than of the O cultures of both nitrogen series, suggesting a possible precipitation of both elements as insoluble ferric phosphate.

The formation of other insoluble compounds of iron and phosphorus is suggested by the results of the chemical analyses. The calculations in table IX are an attempt to show certain possible relationships between phosphorus and iron which might have affected their distribution in the roots of the plants of the different cultures.

The calculations in table IX show that the ratio of iron to phosphorus in the F cultures was 0.278 and 0.415 in the N-n and A-n series, respectively. The corresponding percentage values of phosphorus in combination with other bases than iron were 84.4 and 76.5 for the N-n and A-n series, respectively, and indicate that 7.9 per cent. (84.4–76.5 = 7.5) more phosphorus was in combination with other bases than iron. The differences between the

TABLE IX

AMOUNTS OF IRON AND PHOSPHORUS AND RATIOS BETWEEN THESE AND ALSO BETWEEN THE THEORETICAL RATIO (1.77) IN THE ROOTS OF THE DIFFERENT CULTURES AND PERCENTAGE VALUES OF PHOSPHORUS IN COMBINATION WITH BASES OTHER THAN IRON

CULTURES	NITRATE SERIES					AMMONIUM SERIES				
			RATIOS					RATIOS		
	Fe	P	$\frac{Fe}{P}$	$\frac{O}{T}$*	Px†	Fe	P	$\frac{Fe}{P}$	$\frac{O}{T}$*	Px†
	mg./gm.	mg./gm.			%	mg./gm.	mg./gm.			%
Plus iron	0.220	0.0790	0.278	0.1565	84.4	0.0282	0.0680	0.4150	0.2345	76.5
Minus iron	0.0012	0.0460	0.026	0.0147	98.5	0.0003	0.0380	0.0079	0.0045	99.5
O–F					14.5					23.0

* $\frac{O}{T}$; O = observed $\frac{Fe}{P}$ ratio; T = theoretical ration of $\frac{Fe}{P}$ = 1.77.

† Px = phosphorus unaccounted for, that is, in combination with other than Fe or a constituent of root cells; was obtained from $1.0 - \frac{O}{T} \times 100$.

O cultures were insignificant. The precipitation of greater amounts of phosphorus on the roots of the plants of the N-n than of the A-n series suggest that the higher pH values of the former cultures were responsible for the extra amounts of precipitated phosphorus. The data further suggest that the smaller amounts of phosphorus and iron in the leaf and stem sections of the plants of the N-n than of the A-n series of the F cultures were caused by the precipitation of both iron and phosphorus under the high pH conditions of the cultures of the former series. The results indicate, generally, that iron (and in certain cases phosphorus) deficiencies might develop in plants during the absorption of certain nutrients by roots from solutions favoring generation of high pH values and insolubleness of these elements.

Regarding the effects from the amounts of iron in the F and in the O cultures on the accumulation of various nutrient elements in plants, the statistical data in table VIII are of considerable interest. They show that the amounts of dry matter and iron increased while magnesium decreased significantly in the F cultures of both nitrogen series. The amounts of certain other elements were affected differently by iron. For instance, the accumulation of calcium was increased in the plants of the F cultures of the N-n series, but decreased in those of the A-n series. The opposite condition was found in the amounts of ash and potassium which were high in the plants of the O cultures of the N-n series and low in those of the A-n series.

Summary

1. Growth of *A. comosus* in minus-iron solution cultures (with traces of Fe from C.P. nutrient salts) was approximately as good as it was in plus-iron cultures when supplied with ammonium-nitrogen salts as a source of nitrogen. In solution cultures with nitrate-nitrogen salts, however, the growth in the minus-iron cultures was appreciably inferior to that in the plus-iron cultures.

2. Average drift of H-ion concentrations during the 2-week intervals between renewal of the nutrient solutions was from pH 6.6 to 4.2 for the ammonium-nitrogen series, and from pH 4.4 to 6.8 for the nitrate-nitrogen series. Precipitation of iron and decrease of its availability to plants was caused by rising pH values, as in the nitrate-nitrogen series. Iron availability was relatively high in the cultures of the ammonium-nitrogen series because of the lowering of pH values.

3. Iron was absorbed at exceedingly low rates from the solution cultures of the nitrate-nitrogen and ammonium-nitrogen series. Although the amounts of iron translocated from the roots to the leaves were greater in the plants of the ammonium-nitrogen than of the nitrate-nitrogen series, those precipitated on the roots were relatively high in both series.

4. Amounts of chlorophyll (to be reported in a subsequent publication), rather than of iron were observed to constitute a better criterion for measuring the response of plants to different iron applications.

5. Phosphorus was found to increase slightly the amounts of iron precipitated on the roots of plants.

6. More manganese was found in the roots and less in the leaves of the plants of the plus-iron than of the minus-iron cultures, suggesting possible precipitation by iron.

7. The amounts of ash and particularly of potassium were higher on a percentage but not on a per plant basis in the plants of the minus-iron than of the plus-iron cultures of the nitrate-nitrogen series. In the ammonium-nitrogen series the differences were small and in some cases the results were reversed.

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LITERATURE CITED

1. BALLS, ERIC G. Biological oxidations and reductions. Ann. Rev. Biochem. Stanford University Press, California. **11**: 1–25. 1942.

2. BERENBLUM, I., and CHAIN, E. An improved method for the colorimetric determination of phosphate. Biochem. Jour. **32**: 295–298. 1938.

3. CONRAD, J. A comparative study of Student's method and Bessel's formula. Jour. Amer. Soc. Agron. **22**: 949–962. 1930.

4. FISHER, R. A. Statistical methods for research workers. Oliver and Boyd, Edinburgh. Fourth rev. ed. 1933.

5. FOLLETT-SMITH, R. R., and BOURNE, C. L. C. The uptake of minerals by pineapple plants at different stages of growth. Agr. Jour. British Guiana **7**: 17–20. 1936.

6. GILE, P. L. Chlorosis of pineapple induced by manganese and carbonate of lime. Science n. s. **44**: 855–857. 1916.

7. ——————. Relation of calcareous soils to pineapple chlorosis. Puerto Rico Agr. Exp. Sta. Bull. 11. 1916.

8. GIRARD, P. Essai d'un schema physique de l' hemipermeabilite des cellules vivantes aux ions. Compt. Rend. Acad. Sci. (Paris) **159**: 376–379. 1914.

9. ——————. Mecanisme electrostatique de l' hemipermeabilite des membranes aux ions. Compt. Rend. Soc. Biol. **76**: 839–841. 1914.

10. HAYNES, DOROTHY. The action of salts and non-electrolytes upon buffer solutions and amphoteric electrolytes and the relation of these effects to the permeability of the cell. Biochem. Jour. **15**: 440–461. 1921.

11. HILL, R. A method for the estimation of iron in biological material. Proc. Roy. Soc. London **B 107**, 205–214. 1930.

12. HORNER, JOHN M. A study of the composition of pineapple plants at various stages of growth as influenced by different types of fertilization. Exp. Sta. Assoc. Hawaiian Pineapple Canners, Bull. 13. 1930.

13. JOHNSON, MAXWELL O.   Manganese chlorosis of pineapples: its cause and control.   Hawaii Agr. Exp. Sta. Bull. 52.   1–38.   1924.

14. KELLEY, W. P.   The function and distribution of manganese in plants and soil.   Hawaii Agr. Exp. Sta. Bull. 26.   1–56.   1912.

15. LOVE, H. H.   A modification of Student's table for use in interpreting experimental results.   Jour. Amer. Soc. Agron. 16: 68–73.   1924.

16. OLSEN, CARSTEN.   Iron absorption and chlorosis in green plants.   Compt. Rend. trav. Lab. Carlsberg, Serie Chim. 21(2): 15–52.   Copenhague.   1935.

17. SAYWELL, L. G., and CUNNINGHAM, B. B.   Determination of iron.   Colorimetric O-phenanthroline method.   Ind. & Eng. Chem. Anal. Ed. 9: 67–69.   1937.

18. SIDERIS, C. P.   Colorimetric microdetermination of cobalt and potassium.   Ind. & Eng. Chem. Anal. Ed. 9: 145.   1937.

19. —————.   Colorimetric microdetermination of manganese.   Ind. & Eng. Chem. Anal. Ed. 9: 445.   1937.

20. —————.   Colorimetric microdetermination of magnesium.   Ind. & Eng. Chem. Anal. Ed. 12: 232.   1940.

21. —————.   Improvement of the formaldoxine colorimetric method for manganese.   Ind. & Eng. Chem. Anal. Ed. 12: 307.   1940.

22. —————.   Improvements in the colorimetric microdetermination of phosphorus.   Ind. & Eng. Chem. Anal. Ed. 14: 762.   1942.

23. —————, KRAUSS, B. H., and YOUNG, H. Y.   Assimilation of ammonium and nitrate by pineapple plants grown in nutrient solutions and its effects on nitrogenous and carbohydrate constituents.   Plant Physiol. 13: 489–527.   1938.

24. —————, —————, and —————.   Distribution of nitrogenous and carbohydrate fractions and other substances in exposed and covered pineapple sister shoots.   Plant Physiol. 15: 225–255.   1940.

25. STEWARD, G. R., THOMAS, E. C., and HORNER, JOHN.   The comparative growth of pineapple plants with ammonia- and nitrate-nitrogen.   Soil Sci. 20: 227–241.   1925.

26. WADLEIGH, C. H., ROBBINS, W. R., and BECKENBACH, J. R.   The relation between the chemical nature of the substrate and the degree of chlorosis in corn.   Soil Sci. 43: 153–173.   1937.

FLAGELLATES OF LATICIFEROUS PLANTS[1]

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(WITH FOURTEEN FIGURES)

Introduction

Since flagellates have been reported to be present in a large number of laticiferous plants, the increased interest in the possibility of using these plants for sources of rubber, and their introduction into the agriculture of new regions of the United States and other American countries has made desirable further studies on these organisms. After the statement was published by HEGNER (2) that flagellates had not been found commonly in economic rubber plants, their occurrence in *Taraxacum kok-saghyz* has been reported by ISAKOVA (4). A survey of the literature shows that they have been reported for a number of rubber plants in their native habitats. The introduction of such plants into extensive culture may make more frequent the usually sporadic occurrence of these flagellates, provided their insect vectors also become more prevalent in mass cultivation of the host plants used for rubber production. Without the presence of natural vectors, infestation may be present only in plants vegetatively propagated since the flagellates have not been shown to be transmitted through seed.

Some of the laticiferous plants have been suspected of being reservoirs for flagellates that produce animal and human infections causing tropical ulcers. Various skin ulcers of the Brazilian type of Leishmaniasis are said to be very common among persons who are engaged in clearing uncultivated woodlands, as in the clearing of rubber plantations (7). The transmission of plant flagellates to mammalian hosts, however, has been only occasionally successful. STRONG (9) found flagellates morphologically identical in the latex of a Euphorbia, in a hemipterous insect feeding on this plant, and in a lizard feeding on the insects; he produced a skin ulcer in a monkey by injecting the intestinal contents of the lizard. Non-flagellate forms from the ulcer were morphologically similar to Leishmania.

Effects of flagellates on host plants

Some authors have reported symptoms of the hosts that indicated a pathological condition in flagellate infested plants. HOLMES (3) has indicated that *Herpetomonas elmassiani* in the latex of *Asclepias syriaca*, produces little or no symptoms of pathogenicity. Heavy infestations make the latex somewhat watery. WENYON (10) states that starch and other latex granules may disappear, causing a watery appearance of the latex. It is difficult to conclude that the presence of such large numbers of flagellates as occur in latices, can have no effect on the constituents of the latex. From experience in the transplanting of infected and healthy plants of the same

species, a weakness of the infected plants seems to result in decreased transplant survival. This was especially noticeable in Chamaesyce species in which the flagellates were present in great numbers. Also, infested plants did not survive well the removal of leaves and flower clusters for the purpose of examining and obtaining latex for culture of the flagellates in artificial media.

Occurrence of flagellates in laticiferous plants

The authors have taken as the criterion of positive infestation of a plant by flagellates, the identification of motile flagellates in microscopic amounts of fresh, undiluted, and uncontaminated latex and the confirmation by a second observer. If their motion was too rapid, or the latex too opaque to identify the presence of flagella in motion, a mount stained with Wright's blood stain was used to confirm the presence of a flagellum on the organism. Such stained mounts have been preserved to authenticate the identification. Living, infested plants were maintained for several months to facilitate study of variations of the flagellates and their effects on the host plants. Moderate dilution of latices with distilled water caused swelling and plasmoptysis within 10 to 30 seconds. Sterile normal salt solution (NaCl 0.8 per cent.) causes some swelling, but maintains motility of the flagellates for several hours. Mounts in normal saline solution should not be used for making dimensional measurements. The drying incident to the use of solvents in Wright's blood stain decreases the diameters of thick-bodied forms. The dimensions of body and flagellum are changed by longitudinal division. Latices in which many dividing flagellates are seen, generally show many thin forms.

Flagellates have been reported previously in 28 species of the Euphorbiaceae (9), 5 species of 4 genera of the Asclepiadaceae, 5 species of 4 genera of the Apocynaceae, 1 species of the Sapotaceae, and 3 species of Ficus of the Urticaceae. In the Compositae they have been reported in *Taraxacum kok-saghyz* (4). The reliability of a number of identifications has been questioned because cell inclusions, elongated nuclei, and protein aggregates, may have a flagellate-like appearance, especially in stained sections. Of the commercial rubber-producing plants, flagellates have been reported only in *Cryptostegia grandiflora, Funtumia elastica,* and *Taraxacum kok-saghyz.* At the U. S. Plant Introduction Garden, Coconut Grove, Florida, in the neighboring areas, and on the Keys, 12 species of plants were found that have not been reported as hosts of the flagellates.

Flagellates of somewhat different morphological characters and different host of origin were found in the families Euphorbiaceae and Asclepiadaceae; in the genera Poinsettia (SMALL's classification), Chamaesyce, and Funastrum; and in 14 species, as follows: *Poinsettia heterophylla* (L.) Small, *P. cyathophora* (Murr.) Small, *P. pinetorum* Small, *Chamaesyce adenoptera* Small, *C. buxifolia* (Lam.) Small, *C. deltoidea* (Engelm.) Small, *C. hirta* (L.) Millspaugh, *C. hypericifolia* (L.) Small, *C. hyssopifolia* (L.) Small,

C. maculata (L.) Small, *C. mathewsii* Small, *C. polygonifolia* (L.) Small, *C. tracyi* Small, and *Funastrum clausum* Jacq.

In the genus Poinsettia, flagellates had previously been reported in *P. heterophylla*. *P. pulcherrima*, the common ornamental shrub, showed no flagellates in the white, pink, red, or double red varieties. Hemipterous insects were seen travelling between *P. heterophylla* and *P. pulcherrima* and seem commonly to feed on both when in close proximity.

Of the genus Chamaesyce, all of the species found in a limited survey of the region of South Florida showed flagellates present in certain individuals. *C. hirta, C. hyssopifolia, C. hypericifolia, C. adenoptera, C. conferta, C. maculata,* and *C. mathewsii,* are extensively infested. *C. buxifolia* is usually infested, *C. tracyi* less commonly, and *C. deltoidea* rather rarely infested. The genus Chamaesyce is very large, and other regions will need to be searched to find if flagellates may be present in all the species of it.

Hundreds of specimens from other genera of laticiferous plants were examined without finding flagellates. These are reported as genera, and indicate that although flagellates were commonly found in the species here reported from the same locality, these genera did not become infested at the U. S. Plant Introduction Garden : Adenium, Alstonia, Asclepias (3 species), Calonyction, Castilla, Catharanthus, Convolvulus (3 species), Croton, Cryptostegia (3 species), Echites, Euphorbia (4 species), Ficus (20 species), Funtumia, Hevea, Ipomea (2 species), Landolphia, Manihot (2 species), Masconhazia, Metastelma, Musa (2 species), Pedilanthus, Phyllanthus, Rhabdadenia, Rhus (2 species), Ricinus, Stillingia (2 species), Strophanthus (2 species), Taraxacum (2 species), and Ursola.

Infected plants of the species examined growing in their natural habitats show no visible symptoms of disease, decreased vigor or green color. Infection can be determined only by microscopic examination for the flagellates. Watery latex cannot be used as a criterion of infection. Flagellates were found in very viscous, creamy latex of *Funastrum clausum,* growing in a dry soil. In the U. S. Plant Introduction Gardens of Coconut Grove, Florida, *Chamaesyce conferta* growing in cracks of bituminous pavement showed general infestation in dry weather; after a heavy rain, flagellates were hard to find. *Chamaesyce hypericifolia* growing in coral rock in full sunlight showed a high incidence of infestation whereas large vigorous plants growing in shade on moist glade land immediately adjacent showed no flagellates. *Poinsettia heterophylla* showed infestation of one branch tip of a plant 8 feet tall, but no flagellates in latex from another branch. A distance of 4 feet of stem separated the tips of these branches.

The flagellates frequently disappear from the latex of petioles of leaves and from the peduncles of flower clusters. After a rain of several hours, a potted plant (*P. cyathophora*) that in days previous thereto had shown numerous flagellates, failed to show their presence in any petiolar latex, whereas the flagellates remained in a plant whose leaves were protected from this rain.

Fɪɢ. 1.　*Chamaesyce adenoptera* flagellate (× 1220).

Fig. 2.   *Chamacsyce buxifolia* flagellate [involution forms (×1220)].
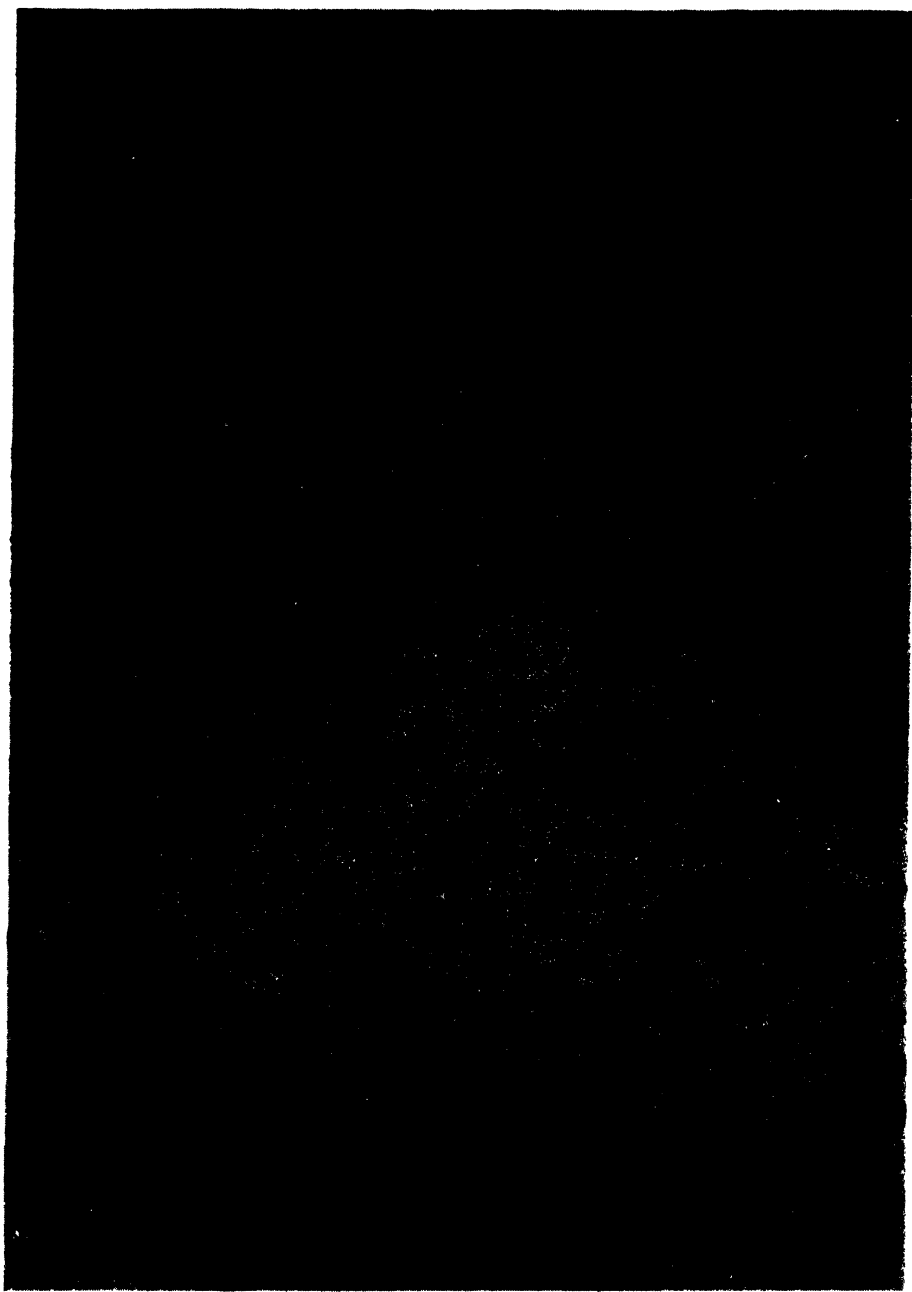
FIG. 3. *Chamaesyce conferta* flagellate (× 1220).
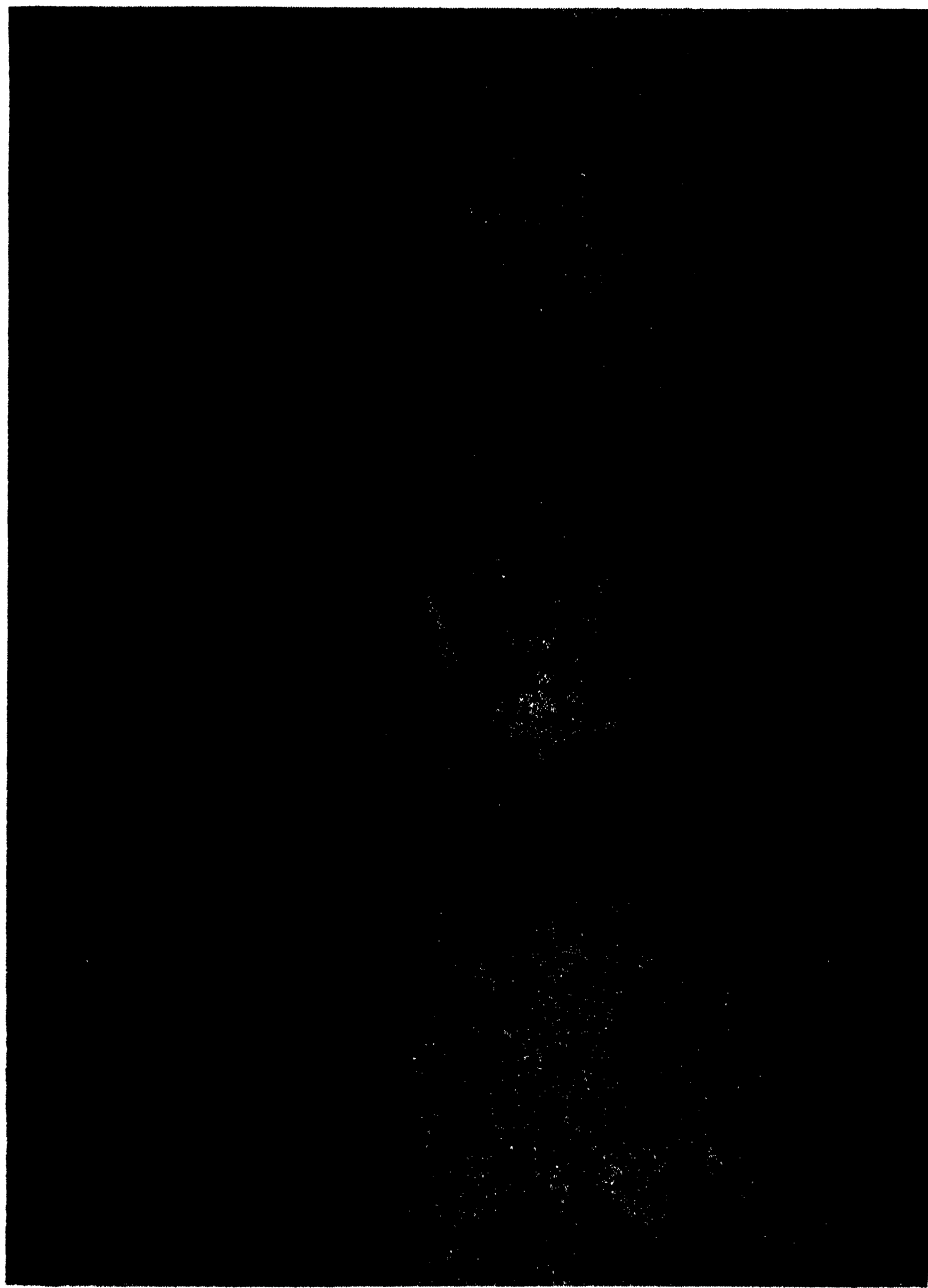
Fig. 4. *Chamaesyce deltoidea* flagellate (× 1220).
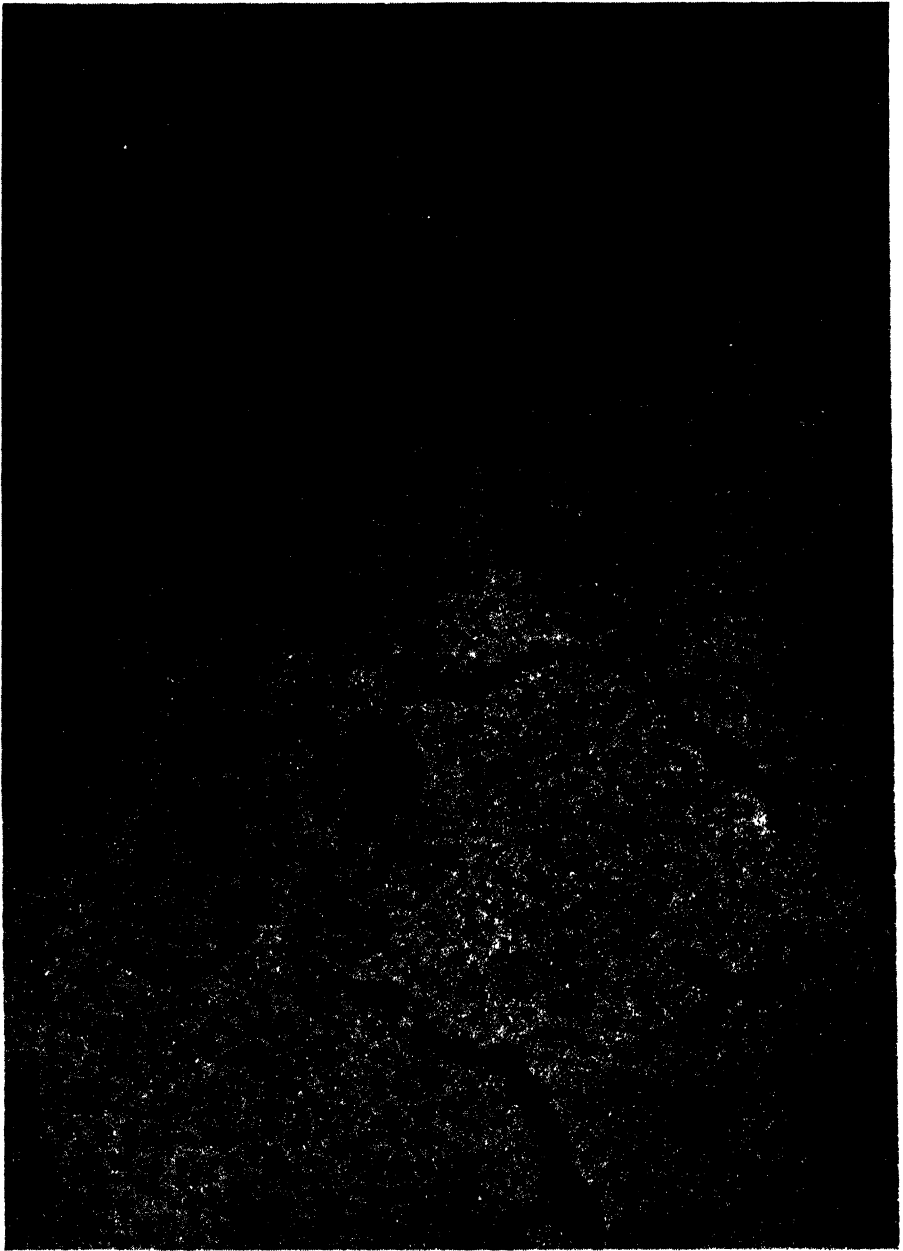
FIG. 5. *Chamaesyce hypericifolia* flagellate (× 1220).

FIG. 6.   *Chamaesyce hyssopifolia* flagellate (× 1220).

FIG. 7. *Chamaesyce maculata* flagellate (×1220).

FIG. 8. *Chamaesyce mathewsii* flagellate (×1220).
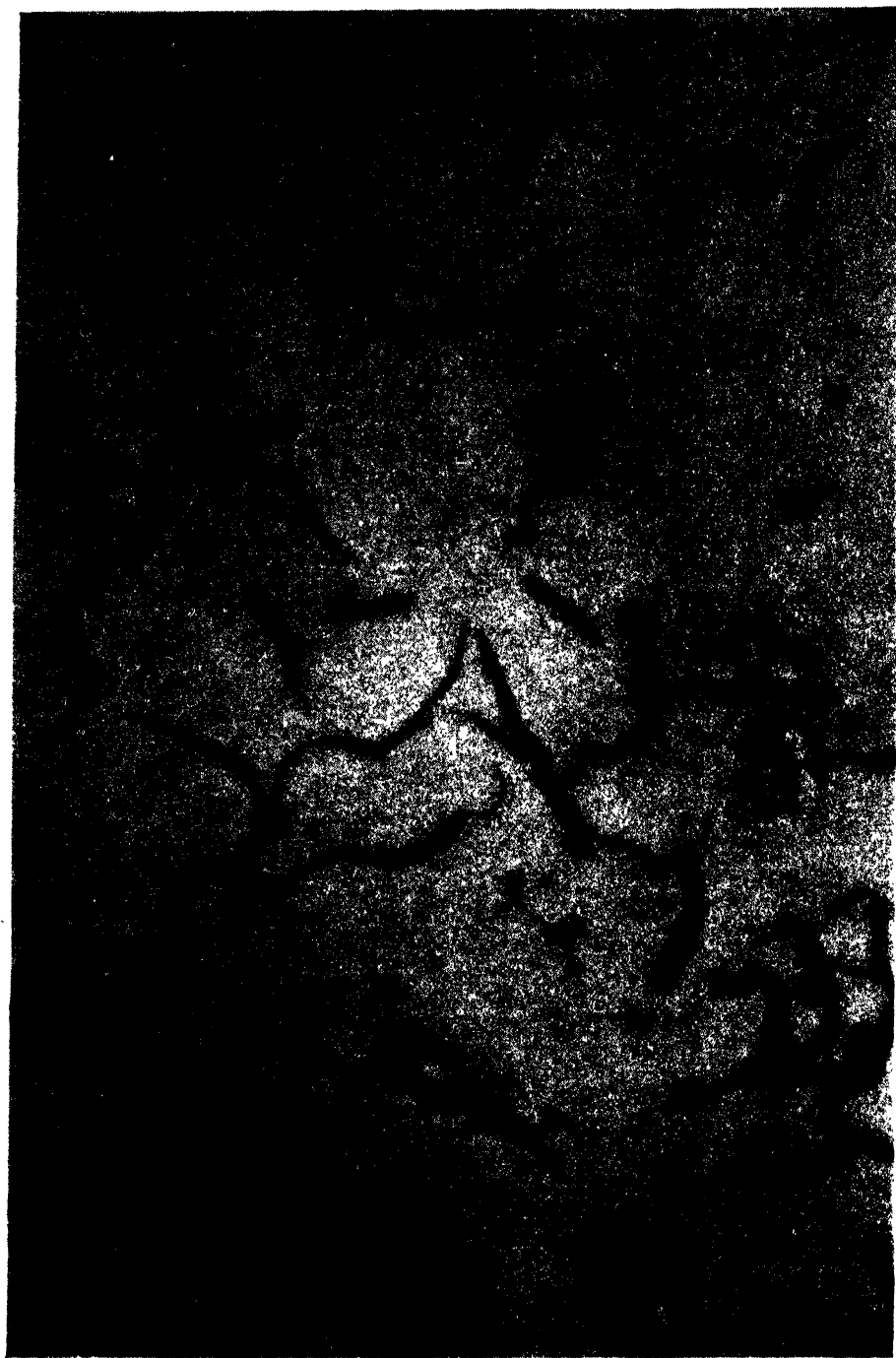
FIG. 9.   *Chamaesyce polygonifolia* flagellate ($\times 1220$).
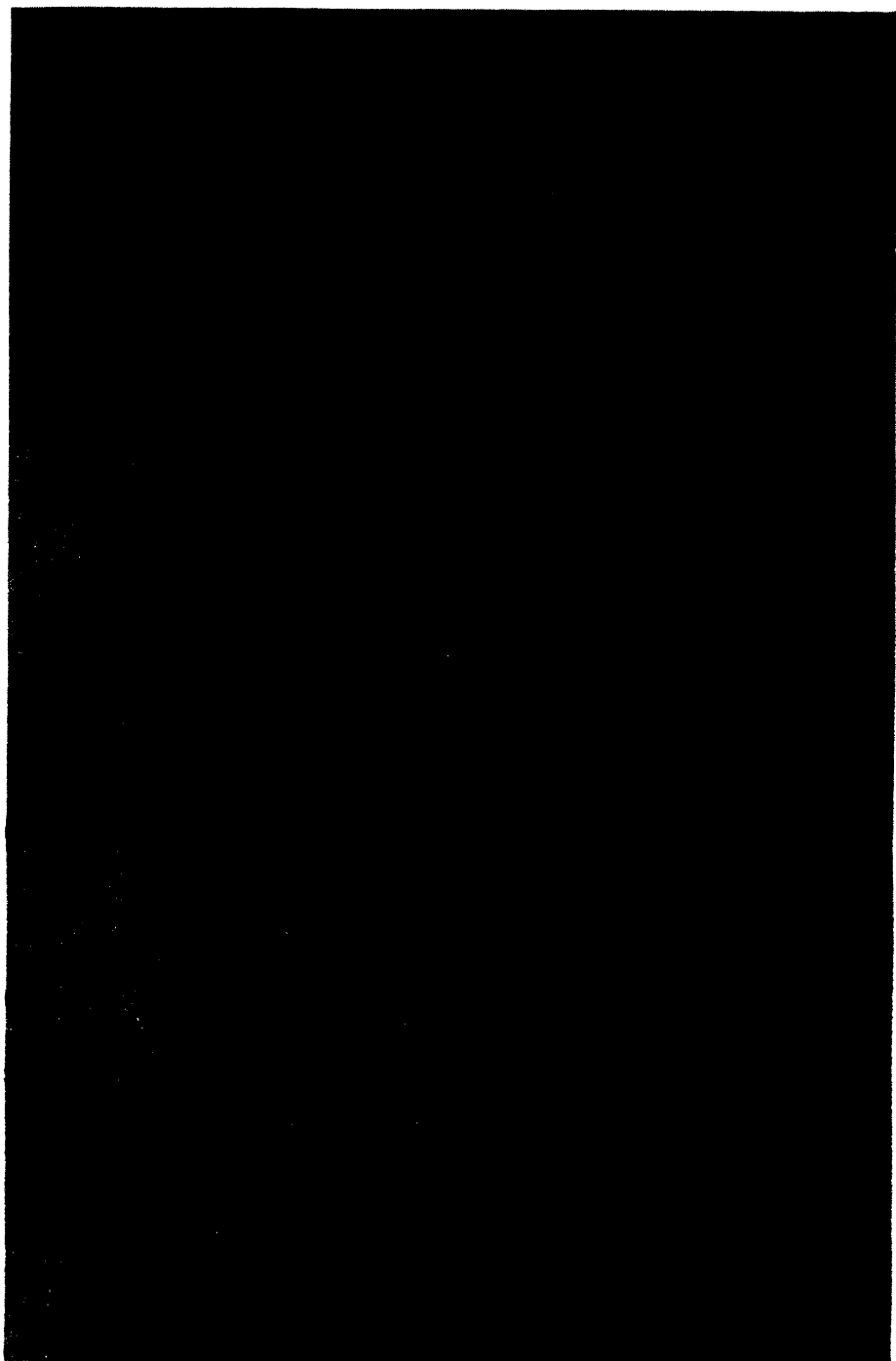
FIG. 10.   *Chamaesyce tracyi* flagellate (× 1220).
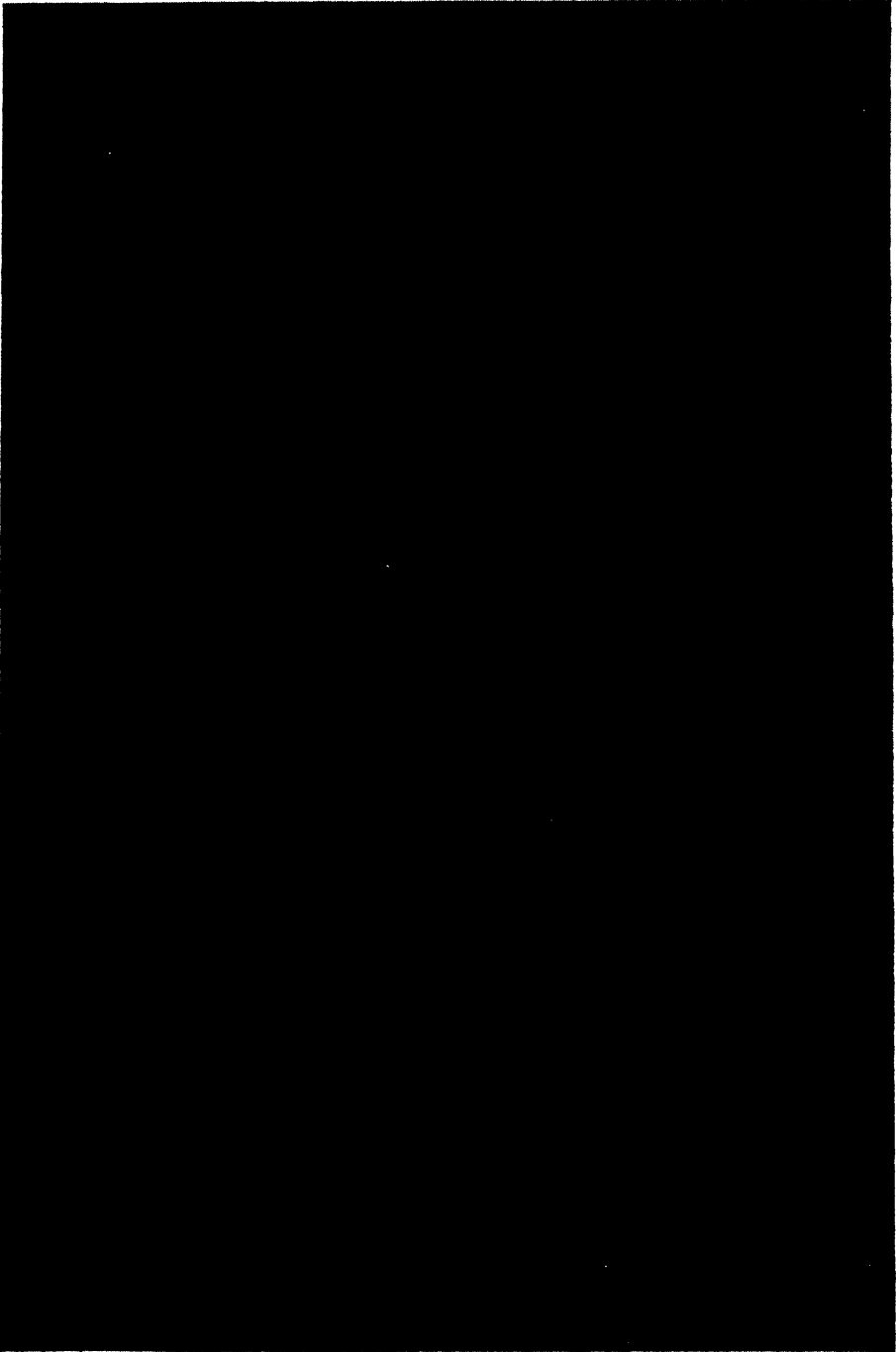
FIG. 11. *Funastrum clausum* flagellate (× 1220).
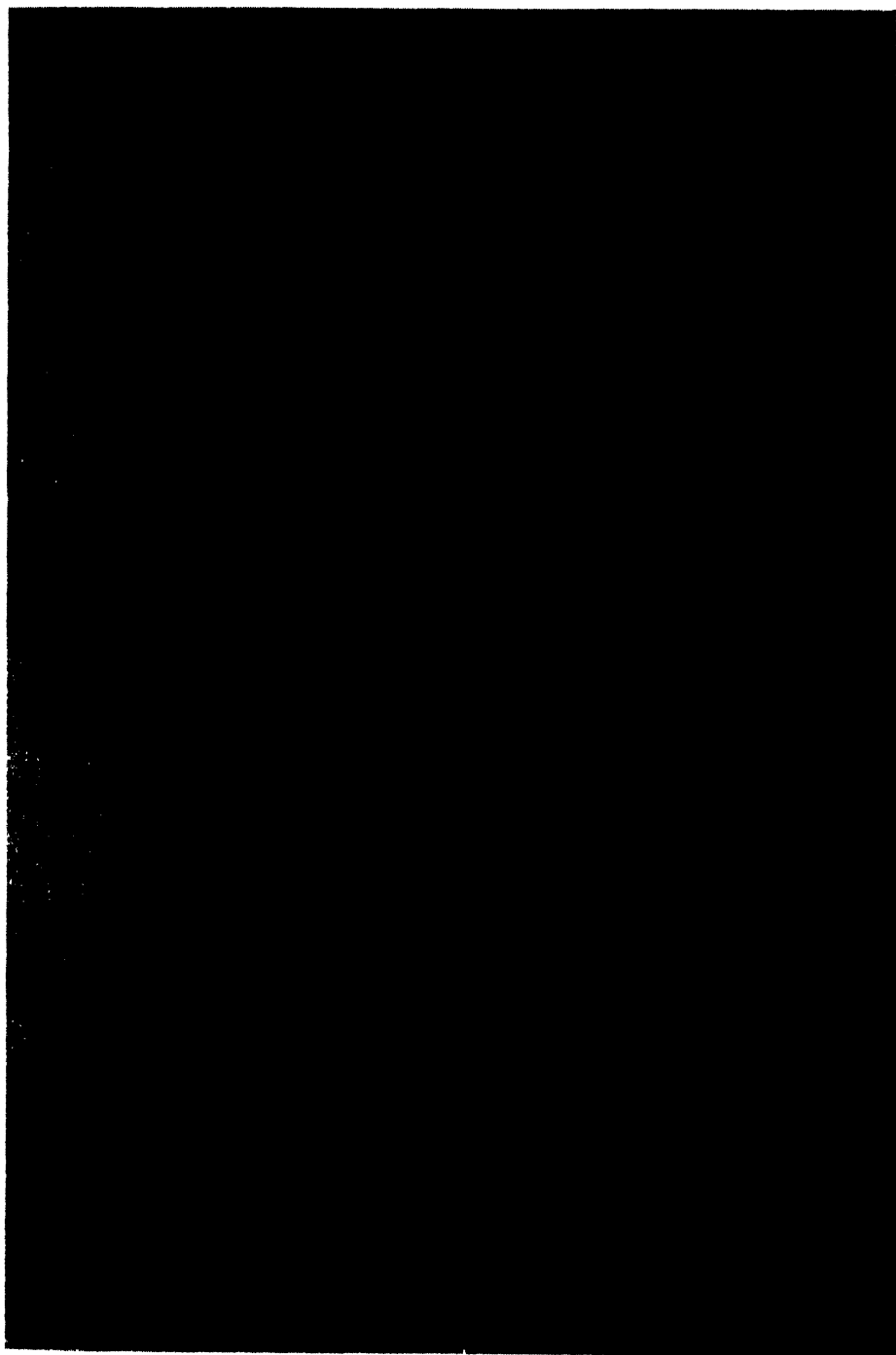
FIG. 12.   *Poinsettia cyathophora* flagellate (×1220).
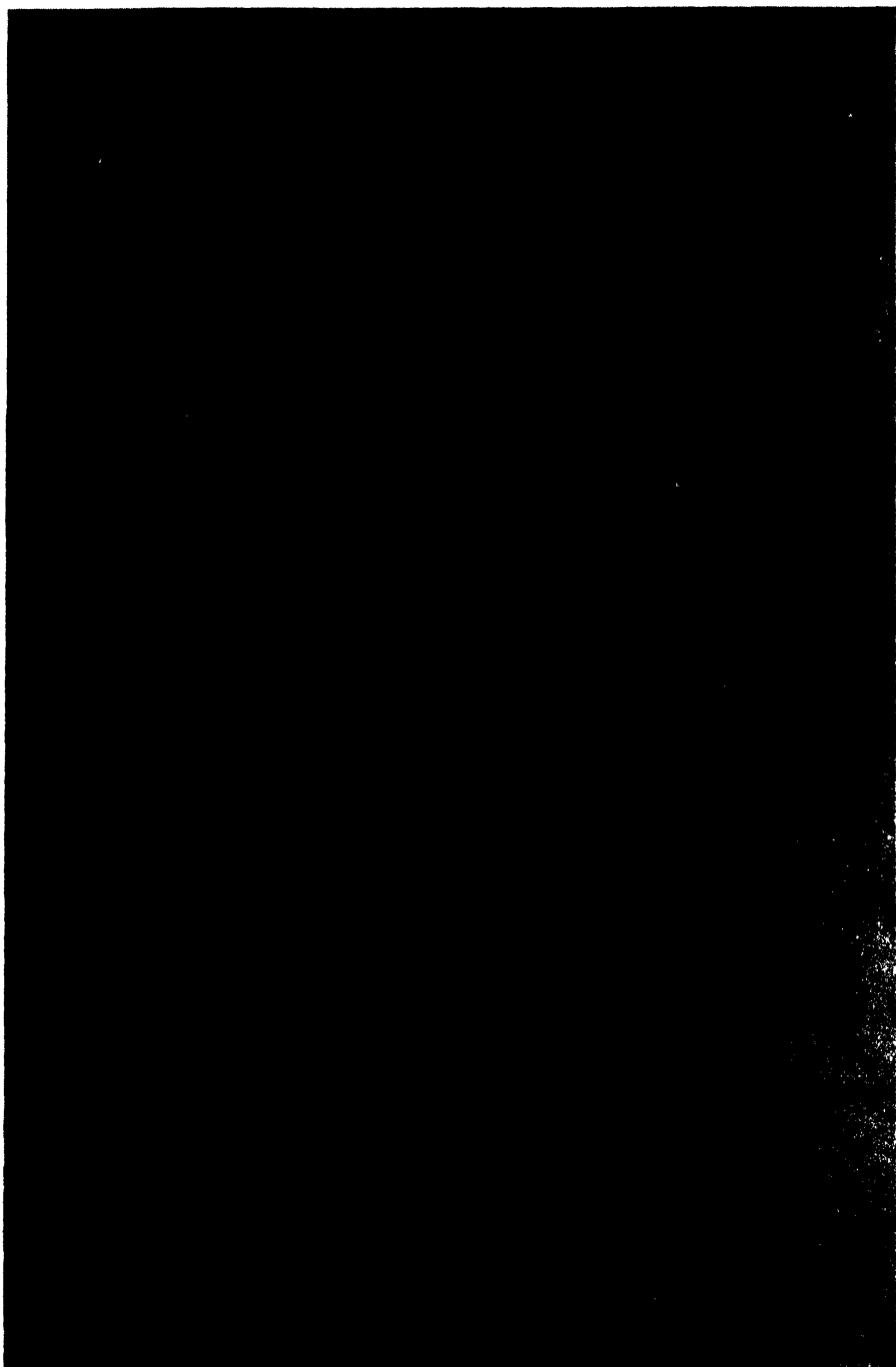
FIG. 13. *Poinsettia heterophylla* flagellate (× 1220).
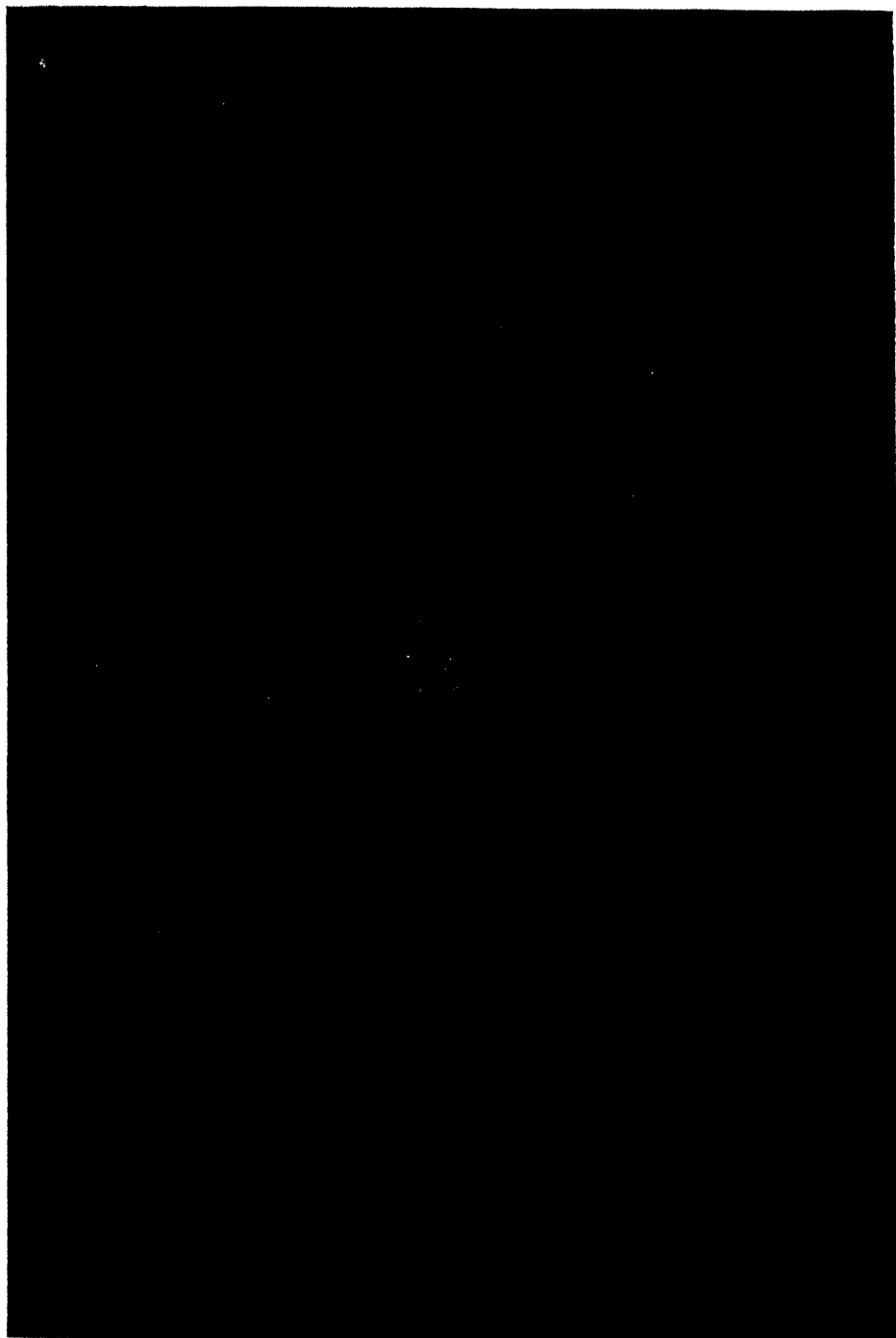
FIG. 14.   *Poinsettia pinctorum* flagellate (×1220).

Flagellates are difficult to count for actual incidence (to obtain a picture of their normal distribution in a plant) on account of the mobility of the latex and also of the organisms themselves. Crude estimates on petiolar latex of *Poinsettia heterophylla* show greatest abundance in the red flag leaves at the tips of the branches, decreasing toward the base of the plant. In latex from the successive nodes of the stem, the greatest number was found in the latex of terminal nodes with decreasing numbers toward the base, then an apparent increase in numbers above the soil level.

Funastrum clausum, infested vines 50 to 70 feet long, showed abundant flagellates in the green tips but none in the brown bark covered stems above the point of rooting.

Culture of latex flagellates

Attempts have been made to study numerous latex flagellates in artificial culture. Upon reviewing previous work on these microorganisms, HOLMES (2) states: "Cultures of latex flagellates are difficult to establish. Suitable media may be inoculated with the organisms many times without the formation of successful colonies."

FRANCHINI (1) and NIESCHULZ (6) used plates of NÖLLER's horse blood agar in the cultivation of a flagellate from a plant of *E. cereiformis*. This medium is a nutrient broth containing agar and glucose (1 per cent.) to which an equal part of defibrinated horse blood is added before pouring plates.

MIGONE (5) used human blood agar to culture a member of the genus Herpetomonas from the host plant *Morenia odorata*. NOGUCHI (8) mentions the difficulty of adaptation of the flagellates to the media and obtained pure cultures of plant flagellates only on leptospira medium and only three times among many attempts. He obtained cultures of 2 species of flagellates from plant latices: *Hepetomonas oncopelti* from *Asclepias syriaca* and *A. nivea*; and *H. lygaeorum* from *A. syriaca*.

Culture of the flagellates of all of the genera was attempted on many hundreds of media modifications of those already successfully used for flagellates of animal diseases. Although survival of motile forms was seen for 4 to 5 days in the most favorable of these media, mass multiplication was obtained only in a rich medium injected into live coconuts containing their natural milk, and in the same medium in flasks to which Hevea latex was added.

For inoculation, petioles of leaves, peduncles of flower clusters, or tips of stems were first swabbed with Lugol's iodine solution, or tincture of iodine. This treatment did not kill the flagellates within the laticiferous system if the solvent was allowed to dry before cutting into the laticiferous tubes. On cutting the plant with a flamed scalpel or knife, the latex exuded in drops which were collected in a flamed platinum loop dipped into the sterile medium before touching the latex.

Transfer to normal salt solution was used in some cases to obtain a quantity of inoculum that later was transferred to the medium by loop or

into coconuts by sterile glass syringe. In inoculating coconuts the best procedure was to remove the husk and sterilize the largest of the embryo spots on the shell with tincture of iodine. A hot needle was then used to puncture the soft tissue of the coconut embryo. Injection of medium and inoculum from the syringe generally did not require removal of milk from the cavity in nearly ripe fruit. In green fruit the milk cavity usually exhibited a strong hydrostatic pressure, and some milk must be removed to allow additions. The removal of the embryo plug by a hot cork borer did not generally give preparations as free from contamination as did needle puncture. A wad of cotton containing iodine tincture was then placed over the inoculation point and covered with adhesive tape. In needle punctures, the endosperm swelled up, closing the puncture, and preventing drying. In the removal of embryo plugs there was more loss of moisture through the cotton plug and more contamination from fungi growing over the shell.

The best basic medium was:

Beef extract (Difco)		1.5 gm.
Peptone (Difco)		2.5 "
Yeast extract (Difco)		2.6 "
Glucose	...	3.3 "
Na acetate		3.3 "
Distilled H_2O		1000 ml.

Fresh rabbit's blood (5 per cent.) was added aseptically from the marginal vein of the ear. The final pH was 7.0.

The acidity of this medium was varied from pH 3.0 to 8.0. The osmotic pressure was varied by adding 0.0 to 0.9 per cent. sodium chloride and the cultures were incubated under aerobic and anaerobic conditions at temperatures ranging from 20° to 37° C. From a study of these conditions with the above medium it was found that aerobic conditions at pH 7.0 with no added sodium chloride and a temperature of 30° C. gave most favorable results. With this medium and these conditions flagellates were demonstrated alive and motile after periods of 9 to 12 days.

Attempts were made to improve this medium by the addition of sterile (Berkefeld filtered) preparations of the following growth factors with amino acids, and cyclic nitrogen bases at levels equal to or slightly higher than those found effective for other microorganisms: thiamine, riboflavin, pyridoxine, pantothenic acid, nicotinic acid, para-amino benzoic acid, biotin, folic acid (concentrate), inositol, glutamine, asparagine, tryptophane, glutamic acid, arginine, cystine, glycine, lysine, histidine, threonine, proline, alanine, valine, hydroxyproline, methionine, β-alanine, leucine, cysteine, adenine, guanine, uracil, and xanthine.

Modifications of the above media, as well as several other media containing blood, have been tested for their ability to support the growth of latex flagellates. Also rabbit hemoglobin, fresh and pasteurized rabbit

serum, and beef blood were used to replace the rabbit blood in the above medium, but without good growth.

EGG MEDIA

Fertile and non-fertile eggs were inoculated directly into the yolk and albumen with latex flagellates. The results were negative after incubation periods of a few days to several weeks. Several other types of egg media were tested. These include: modified Boeck's Locke's medium, Hogue's egg medium, and Schneider's and Boeck's Locke's egg medium plus blood serum. The results were negative.

DIFCO MEDIA

Numerous media prepared by Difco for the culture of fastidious micro-organisms were tested for their ability to support the growth of latex flagellates. These included: veal infusion, brain-liver-heart, brain-heart, cabbage infusion, blood-base-agar plus rabbit blood, wort agar, egg-meat broth, and potato infusion. These media were tested alone and in combination with rabbit blood and the growth factors, amino acids, and nitrogen bases listed above. Results were negative.

COCONUT CULTURE

Coconut milk has been used by previous workers in attempts to culture flagellates. Injection of media with flagellates into live coconuts has worked with some success. The coconuts were prepared as described above. The media and the inoculum were injected into the coconuts through the sterilized embryo spots. The pH inside the coconuts was raised from 5.0–5.5 to 7.2 by injecting sterile $M/1$ K_2HPO_4, and sterile supplements of various types were added. The inoculum consisted of a suspension of latex organisms in sterile saline (0.8 per cent.). Using this technique it was possible to demonstrate the presence of numerous motile flagellates after 30 days in a coconut which had been fortified with the rabbit blood medium listed above. Sub-cultures from these coconuts failed. To other coconuts adjusted to pH 7.2 with phosphate buffer, the mixture of growth factors, amino acids, and nitrogen bases listed above was added. No growth was obtained in these media.

CULTURE IN HEVEA LATEX

In the above basic medium with fresh unsterile Hevea latex added, cultures of flagellates from *Funastrum clausum* with rapid multiplication were obtained. The flagellates grew more rapidly than the contaminants at 37° C. and after 12 hours were very numerous and actively motile. The flagellates from *Funastrum clausum* grew and divided into single individuals and also formed long chains of 20 to 30 flagellates that moved with a spiral motion. These chains were easily broken apart by shaking the flask or in making mounts. After 24 hours the flagellates had become immobile or disappeared and the medium was heavily contaminated by motile and

non-motile bacteria, spirillum, and coccus forms. Subculture after 12 hours was attempted in the same basic medium to which was added the serum obtained by precipitating the rubber from fresh Hevea latex with the least amount of acetic acid to obtain a serum separation, neutralization of the serum with NaOH, and passing through a Berkefeld filter. No flagellates grew but contaminants were numerous. Inoculation of a similar sterilized preparation with Hevea latex serum with fresh inoculum from Funastrum gave no growth of flagellates.

In examining latices for fluorescence, an all quartz illumination system was used with light from the G.E. AH4 100-watt mercury arc in clear Corex glass. Flagellates exposed on a quartz slide to the intense ultraviolet and visible light transmitted by this system were very active after 4 hours. Hence the organism seems insensitive to ultraviolet and visible light. Attempts to grow the flagellates in pyrex flasks in sunlight using several nutrient media were not successful.

It is apparent from these cultures that the latex flagellates are difficult to culture and require essentially media derived from live tissues. To obtain such in a sterile condition one is limited to the use of coconuts since it is practically impossible to obtain other sterile plant latices in quantity. NOGUCHI (**6**) has reported that the flagellates of *Euphorbia braziliensis* and *Asclepias curassavica* will not grow in sterile coconut milk media *in vitro*. This is confirmed. A number of reports of culture of these organisms seem to be questionable. Perhaps survival in the media for a few days rather than actively dividing cultures were obtained.

Descriptions

All the photographs (figs. 1 to 14) are at the same magnification ($\times$ 1220) to facilitate comparisons of flagellate dimensions. The latex flagellates in Poinsettia, Chamaesyce, and Funastrum have a single anterior flagellum, arising anterior to the parabasal body. In Funastrum, the flagellum is very short. In the photographs these are not easily recognized, but in live organisms they can be seen in motion. The flagella in Poinsettia and Chamaesyce are frequently longer than the body. The characteristic movement of the flagellum can be seen in the long ones to be a pulsating, spiralling coil motion from the body passing toward the end. Frequently more than one coil can be seen travelling out on the flagellum, as in a rope that is given quick circular movements by the end held in the hand.

The blepharoplast and nucleus differ in their size, position, and density of staining in the different species of hosts. Some nuclei are large rounded and distinct (*C. buxifolia, C. hypericifolia, C. hyssopifolia, C. mathewsii,* and *C. tracyi*) others are elongated and have indistinct outlines (*C. adenoptera, C. deltoidea,* and *C. polygonifolia*).

Twisted ribbon-like bodies are commonly seen, and the motion of the body is in a spiral path through the latex. In chains of flagellates, the movement of the whole series of flagellates is coordinated, giving a ser-

pentine-like movement and rolling the chain. This movement also is characteristic in the twisted ribbon-like forms. In Funastrum only thin twisted ribbon-like forms with blunt-ended bodies were seen. In other latices these same forms occurred: *C. buxifolia, C. conferta, C. deltoidea, C. hirta, C. mathewsii, C. polygonifolia, C. tracyi;* but less commonly in the Poinsettia species. The commonly long flagellated rounded bodies that taper posteriorly to a tail sometimes of a diameter as small as the flagellum, are seen in all of the species of Chamaesyce and Poinsettia. The method of longitudinal division of the body can be seen in the photograph of *P. cyathophora* latex, where two blepharoplasts, two nuclei, and one flagellum can be seen in the flagellate with the rounded body at the center of the figure. More than two bodies can frequently be seen united at the posterior end in rapidly dividing cultures or latices. In *Chamaesyce buxifolia,* in addition to the flat twisted ribbons and rounded tapering bodies, spherical and distorted bodies are commonly found in active motion by their flagella, or quiescent without flagella. These forms may be produced by abnormal water absorption from rain water or tap water used to water the plants, because this species commonly grows in habits of high salinity near the sea coast. In cultures from *C. buxifolia,* the ribbon like forms with 45 degree spiral turn have blunt ends and survive longer than the other forms.

Summary

Twelve species of plants are newly reported as hosts of latex flagellates. Photographs of the flagellates from different species are given in the text.

The incidence of infestation was variable in Chamaesyce species, but individuals were found infested in all of the species of this genus that were examined.

Incidence of infestation was found variable with habitat, soil, and weather conditions.

Culture for 30 days was obtained in a medium injected into live coconuts and rapid multiplication occurred in a similar medium to which unsterile fresh Hevea latex was added.

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LITERATURE CITED

1. FRANCHINI, G. Sur un protozoaire d' *Euphorbia cereiformis* et sur sa culture. Bull. Soc. Path. Exot. **16**: 642–646. 1923.
2. HEGNER, R. W., and ANDREWS, JUSTIN. Problems and methods of research in protozoology. Chapt. XXIX. FRANCIS O. HOLMES, Protozoa of latex plants. The Macmillan Co., N. Y. 1930.

3. HOLMES, F. O.   Nonpathogenicity of the milkweed flagellate in Mary-land.   Phytopath. **15**: 294–296.   1925.

4. ISAKOVA, A. A.   The relation between higher plants and lower organisms.   I.   On the association of plants containing latex with micro-organisms.   Bull. Akad. Nauk.   (In Russian).   Ser. **7**: 383–395. 1935.

5. MIGONE, L. E.   Flagelados de las plantas.   Rev. Soc. Cient. Paraguay **1**: 35–39.   1922.

6. NIESCHULZ, O.   Zur Morphologie der Kultur formen einer Herpeto-monas aus *Euphorbia cereiformis*.   Centralbl. Bakt. Abt. 2 **61** (11–18): 311–316.   1924.

7. NOGUCHI, HIDEYO.   Leishmania in tropical ulcer, pp. 455–479.   International conference on health problems in Tropical America. United Fruit Co., Boston.   1924.

8. ——————.   Comparative studies of herpetomonads and leishmanias 1.   Cultivation of herpetomonads from insects and plants.   Jour. Exp. Med. **44**: 307–324.   1926.

9. STRONG, R. P.   The investigation upon flagellate infection.   Amer. Jour. Trop. Med. **4**: 345.   1924.

10. WENYON, C. M.   Protozoology.   Vol. 1 & 2.   William Wood & Co., New York.   1926.

COMPOSITION OF THE ROOTS AND STUBBLE OF PERENNIAL RYEGRASS FOLLOWING PARTIAL DEFOLIATION[1]

J. T. SULLIVAN AND V. G. SPRAGUE

(WITH NINE FIGURES)

When the foliar portion of a grass plant is removed by a mower or by a grazing animal, the growth of new tissues is initiated to replace the parts removed. The rate with which the regeneration of new top growth proceeds and the total amount produced are of great importance in forage production. This rate of recovery and total yield, although dependent to a large extent upon external environmental factors, are also influenced considerably by the intensity and frequency of defoliation as determined by management practices. Evidence has been offered by many workers (**5, 9, 10, 11, 21, 23, 24, 26, 29**) that close and frequent clipping or grazing reduces the total yield of tops of forage plants.

Associated with the ability of the plant to produce new growth after cutting is its chemical composition. Plants cut less frequently and less severely have a greater opportunity to synthesize and maintain reserve food materials which are available for the production of new growth than plants more severely grazed. That carbohydrates are important as reserve substances in pasture plant economy has been pointed out by earlier workers (**1, 4, 7, 8, 13, 14, 15, 16, 25, 27, 28**).

The specific carbohydrates which may be considered "reserves" that can be utilized by the plants for purposes of respiration and production of new tissues have not been definitely established. WEINMANN (**27**) considered that sugars and polysaccharides hydrolyzable by hot dilute HCl were lost from the tops of some South African grasses after the time of flowering and were largely recovered in the roots. In another contribution (**28**), the seasonal changes in the composition of roots were described as mainly due to translocation and storage. Sugars and starch (polysaccharides rendered soluble by saliva and hydrolyzed by HCl) were considered of importance but the results with total hydrolyzable polysaccharides were not conclusive and were not reported. In alfalfa, ALBERT (**2**), GRABER, et al. (**8**), and LEUKEL (**13**) considered sugars, dextrins, starch, and hemicelluloses as available carbohydrates and found that the supply of these in the roots was lowered by frequent cutting of the immature tops. Plants in darkness (**2, 8**) produced new top growth while starch disappeared from the roots; total carbohydrates decreased and hemicelluloses increased. According to BUKEY and WEAVER (**4**) there was a marked decrease in the percentage of invert sugar, water-soluble hydrolyzable, and water-insoluble hydrolyzable

material in the underground parts of Andropogon under conditions of severe clipping. McCARTY (**14, 15**) studied the seasonal fluctuations of sugars, starch, and hemicellulose in several range grasses during the growing season. Carbohydrate storage was inversely related to the rate of vegetative growth of the herbage. He concluded (**15**) that sugars and starches (chiefly sucrose and starch) were the more important of the stored carbohydrate foods. The behavior of the acid-hydrolyzable hemicellulose suggested that this carbohydrate was employed largely as structural material. Sugars and starch were also found by McCARTY and PRICE (**16**) to be reduced in quantity in the roots of mountain brome and slender wheat grass if the plants were grazed during the reproductive period and during the normal storage period. MOROZOV (**17**) concluded from a study of brome grass, ryegrass, and fescue that the places of carbohydrate accumulation were the roots, rhizomes, stems, shortened internodes, and leaves. The carbohydrates stored were chiefly inulin-like substances and hemicellulose.

This inulin-like substance, resembling inulin only in that it yields fructose on hydrolysis, is probably the fructosan widely reported in recent years in the grasses and cereals. NORMAN (**18**) has reported its presence in ryegrass (Western Wolths) in amounts as high as 34 per cent. NORMAN and RICHARDSON (**19**) report that it accumulates chiefly in the stem and acts as a temporary reserve, later being utilized for the production of structural constituents. The wide distribution of fructosan in pasture grasses (**3**) and the lack of confirmatory evidence of considerable amounts of starch make it appear probable that fructosan, rather than starch, is an important carbohydrate reserve. The properties of fructosan are such that in much quantitative work it may have been determined and reported incorrectly as starch. It is water-soluble and alcohol-insoluble, and is readily hydrolyzed to fructose by acids. When starch is determined by saliva or other enzymatic reagents in water solution fructosan may go into solution and be hydrolyzed by the subsequent acid treatment that often follows diastatic action. "Dextrin" and "starch" determinations reported in grasses may therefore have been largely of fructosan. This does not apply to such crops as alfalfa in which starch does occur and in which fructosan has not been shown to be present.

Carbohydrates readily change from one form to another so that by ordinary methods of analysis it is impossible to trace the translocation and utilization of any particular one in the plant. As a first step in the study of the relation of carbohydrates to regeneration of growth in grasses it seems desirable to have a knowledge of the carbohydrate changes taking place in the plant immediately after cutting. In this paper are reported the analyses of perennial ryegrass (*Lolium perenne* L.) plants at intervals after partial defoliation.

Materials and methods

The plant material used in this experiment consisted of a single clone of perennial ryegrass. In November, 1940, cuttings were made and planted in

gravel in one-gallon glazed pots, three tillers per pot, and supplied with a complete nutrient solution by automatic irrigation. The temperature of the greenhouse was maintained at 18 to 20° C. and the normal daylight period was increased to 14 hours with mazda lights at an intensity of approximately 75 foot candles. The average intensity of daylight in the greenhouse was 5,000 to 7,000 foot candles. Three replications were grown on three adjoining benches in the same greenhouse; each bench held one complete replication. In order to obtain sufficient material for analysis, three pots in each replication were composited at the time of harvest.

On March 17, 1941, about three months after establishment, the plants were vegetatively vigorous and had reached an average height of 8 inches. At this time the tops were cut with shears at a height of 1.5 inches above the surface of the gravel. The removed tops were dried and the dry weights determined. They averaged 5.40 grams per pot. They were not analyzed chemically.

The remaining stubble and roots were sampled for chemical analysis immediately after cutting. The plants from 3 pots selected at random from each replication were combined and, after washing and removing the gravel, the roots and stubble were separated. These samples were surface dried with paper towels and dropped into boiling alcohol. Later they were partly extracted, dried, coarsely ground, and completely extracted with 80 per cent. alcohol in the usual manner.

Sampling of the roots and stubble was repeated in the same manner 1, 2, 4, 7, 11, 16, 22, 28, and 36 days after cutting. At each date the new leaf growth which had appeared above the 1.5-inch level was removed and its dry weight only was determined. New leaf tissue below the 1.5-inch level was included in the stubble.

Another series of pots was removed to a darkroom immediately after cutting and plants from them were sampled in the same way and on the same subsequent days as were the plants allowed to recover in the greenhouse. Three pots were used to furnish a composite sample at each date for the material in the darkroom but no replication was available.

The alcoholic extracts were made to volume and an aliquot of each was used to determine total solids. The sum of the total solids of the extract and the alcohol-insoluble residue gave the total dry weight of the sample. Another aliquot of the extract, after removal of the alcohol and after clarification, was used for the determination of sugars. Reducing power was determined by a method essentially that of PHILLIPS (22). Fructose was determined by oxidizing aldoses with iodine by a procedure similar to that of NORMAN, WILSIE, and GAESSLER (20) and determining the residual reducing power. Sucrose was determined by hydrolyzing with hydrochloric acid and determining the increased reducing power. No other carbohydrates were believed to be present in the alcoholic extract. A portion of the alcoholic extract was used for a qualitative test for nitrates and the remainder was used for the determination of total nitrogen including nitrates. Nitrates were found only in plants growing in darkness.

The alcohol-insoluble residue was weighed and further ground to pass a 60-mesh sieve. Aliquots were used for the following determinations:

An extract of the residue was obtained by heating a 1-gram portion with 0.25 per cent. oxalic acid for 1 hour at 80° C., neutralizing with barium hydroxide, filtering, and clearing. Total reducing power and fructose were determined. The fructose was considered as having been derived from fructosan and is so reported, but calculated as fructose.

Cellulose was determined on another 2-gram portion by a modification of the method of CRAMPTON and MAYNARD (6).

A 1-gram portion was treated with normal sulphuric acid in a boiling water bath for 2.5 hours. The residue was removed and washed. The acid liquor and washings were neutralized with $CaCO_3$ and $Ba(OH)_2$, filtered, clarified, and made to volume. Determinations were made on aliquot portions for total reducing power, fructose, and non-fermentable reducing substances. The length of time of the hydrolysis, 2.5 hours, was sufficient to give the maximum yield of non-fermentable reducing power under the conditions of acid concentration and temperature. The non-fermentable reducing power was determined as the residual reducing power after treatment with bakers' yeast. It is expressed as pentosan (calculated as xylose) and the amounts agree closely with the furfural distillation method. Galactose could not be detected qualitatively. The total reducing power of the acid hydrolyzate, after correction for fructose (part of which had been decomposed during the acid treatment) was slightly higher than that of the pentose. The difference, amounting to a low percentage of the sample, was not further characterized. Part of it may be due to theoretical difficulties arising in the calculations of sugar mixtures.

The residue from the normal sulphuric acid treatment was treated with 72 per cent. sulphuric acid for 16 hours at 4° C. and finally with normal sulphuric acid for 5 hours at 100° C. The insoluble residue, after correcting for ash content, was characterized as lignin.

A portion of the alcohol-insoluble residue was used for the determination of total nitrogen by the Kjeldahl method.

Results

INITIAL COMPOSITION OF STUBBLE AND ROOTS

The analysis of variance of the dry weights of tops harvested at the beginning of the experiment (table I) showed that the differences among the

TABLE I

THE ANALYSIS OF VARIANCE OF THE DRY WEIGHTS OF THE TOPS REMOVED FROM
THE PLANTS AT THE BEGINNING OF THE EXPERIMENT

VARIATION DUE TO	D/F	MEAN SQUARE	F VALUE
Dates	9	0.3193	1.12
Replications	3	0.9559	3.34
Dates × replications	27	0.2860	

groups of three pots selected at each of the 10 dates of sampling were not statistically significant. It may be concluded that differential establishment of the plants was not an important factor in conditioning differences obtained at subsequent sampling dates.

The yield and composition of ryegrass stubble and roots, as determined on samples taken immediately after removing the tops, are given in table II.

TABLE II

THE YIELD AND COMPOSITION OF STUBBLE AND ROOTS OF RYEGRASS AT THE TIME OF DEFOLI-
ATION ON MARCH 17, 1941. (EXPRESSED AS PERCENTAGE OF DRY WEIGHT,
EXCEPT FIRST AND LAST ITEMS)

ANALYSES	STUBBLE				ROOTS			
	REPLICATIONS				REPLICATIONS			
	1	2	3	AVE.	1	2	3	AVE.
Total yield in dry matter, in grams per pot*	3.82	4.27	4.17	4.09	6.14	7.26	6.93	6.78
Total constituents accounted for	80.87	80.32	79.51	80.23	78.88	79.57	79.08	79.18
Dry matter soluble in alcohol	23.31	18.32	21.84	21.16	13.26	14.06	13.27	13.53
Fructose	1.93	1.62	1.81	1.79	0.85	0.90	0.89	0.88
Glucose	2.26	1.76	2.00	2.01	0.26	0.27	0.34	0.29
Sucrose	6.37	5.25	6.62	6.08	2.87	2.95	2.85	2.89
Fructosan	14.61	16.17	14.33	15.04	1.92	1.88	1.64	1.81
Aldose obtained from oxalic acid hydrolysis, as glucose	0.80	0.81	0.64	0.75	0.37	0.27	0.38	0.34
Pentosan, as xylose	15.71	16.65	16.27	16.21	23.54	24.02	24.04	24.53
Fermentable aldoses from sulphuric acid hydrolysis, as glucose	4.46	4.28	4.30	4.35	2.02	2.22	1.81	2.02
Cellulose	27.10	28.00	27.02	27.37	32.09	32.06	31.84	32.00
Lignin	3.98	3.95	4.03	3.99	12.04	12.25	12.41	12.23
Alcohol soluble N	0.24	0.16	0.20	0.20	0.09	0.08	0.08	0.08
Alcohol-insoluble N	1.09	0.91	0.91	0.97	0.96	0.93	0.96	0.95
Total N	1.32	1.07	1.11	1.17	1.04	1.01	1.04	1.03
Alcohol soluble N, in percentage of total N	17.75	14.70	17.99	16.81	8.42	8.09	7.94	8.15

* The average dry weights per pot of the tops removed on March 17 were 4.95, 5.09, and 4.89 grams, respectively.

The dry weight of the stubble and its composition depend upon the amount and the structures of the plant included in the tissues below the 1.5-inch level. In this case of vegetatively growing plants, they consisted primarily of leaf sheaths, a small amount of leaf blade tissue, and short stems which probably did not exceed ¼ inch in length. The yield of stubble in dry matter was a little more than half that of the roots. The stubble had greater concentrations than the roots of the more soluble constituents such as sugars, fructosan, and soluble nitrogen and less of the more insoluble constituents such as pentosan, cellulose, and lignin. About 21 per cent. of the total dry matter of the stubble was alcohol-soluble; if fructosan may be included, at least 30 per cent. was water-soluble. At least 15 per cent. of the roots was

water-soluble.   Of the sugars, sucrose was considerably higher than either
glucose or fructose in both the roots and stubble and was higher than fructo-
san in the roots.   Of the two monosaccharides, glucose was the higher in the
stubble and fructose in the roots.   Fructosan was much higher in the stubble
on a percentage basis and also on an actual basis, even though the total dry
weight of stubble was less than that of the roots.   The total constituents
determined, using the factor 6.25 to convert nitrogen to protein, was about
80 per cent. of the dry weight of the stubble and 79 per cent. of the roots.
Corrections for the contamination of lignin and cellulose with protein would
have decreased these figures slightly.

CHANGES IN DRY MATTER AFTER CLIPPING

Dry matter changes of the different plant parts during the days follow-
ing clipping are illustrated in figure 1.   The yields reported, and also all
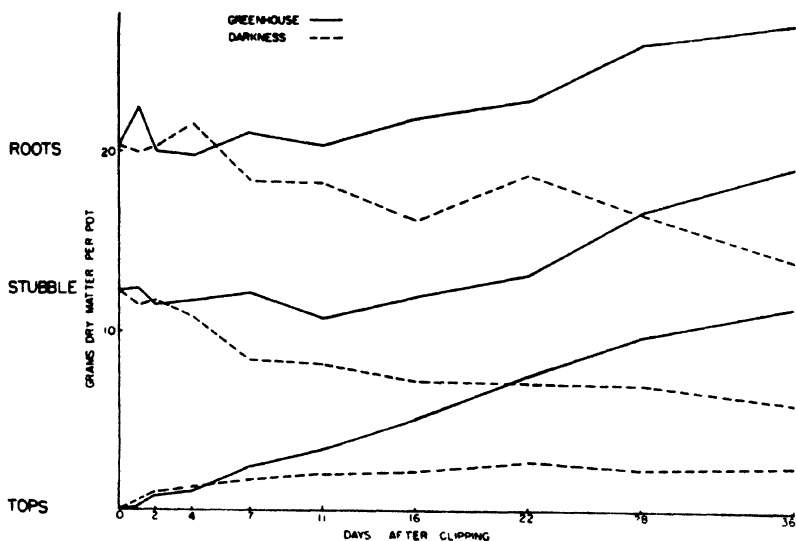
FIG. 1.   Yield of roots, stubble, and new top growth of ryegrass plants growing in
the greenhouse (solid lines) and in darkness (dotted lines) for 36 days after removal of
the tops.

subsequent composition data, are the averages of three replications in the
case of greenhouse plants and are of one replication in plants moved to the
darkroom.   A small but measurable quantity of new top growth above the
1.5-inch level was present on the first day after clipping.   The rate of in-
crease of the tops was fairly constant and the dry matter per pot amounted
to 11 grams at the end of 36 days.   In the dark, the rate of growth was
similar to that of plants in light for about 5 days, after which it decreased.
Total dry matter of regenerated leaves reached a maximum after about 22
days.   During recovery in the greenhouse the stubble growth, including new,
leaf tissue below the 1.5-inch level, fluctuated somewhat, showed significant
losses in total dry matter at several dates, and did not show a definite in-

crease above its original weight until after the 16th day. After 36 days of recovery there had been a net increase in weight of roots, stubble, and leaves amounting to 19 grams per pot. In darkness the loss of dry matter from the stubble was immediate. The roots, amounting to 20 grams of dry matter per pot, did not lose weight in the greenhouse and began increasing in weight after the 11th day, reaching 27 grams after 36 days. The roots in the dark room continually decreased in weight with a minimum of 14 grams on the 36th day after clipping. At the end of this period many of the plants in darkness were undoubtedly dead, though no attempt was made to revive them. Many roots were in stages of decomposition. Dry weight fluctuations in the roots and stubble during the first few days after clipping were probably due to pot variation. As an indication of pot variation the nine pots selected for the first group of samples had averaged 4.93 grams of top growth removed, as compared with total average for all pots of 5.40 grams.

CHANGES IN COMPOSITION AFTER CLIPPING

The changes in composition of the stubble and roots after clipping are illustrated in figure 2 and in all succeeding figures. In figure 2 are illus-
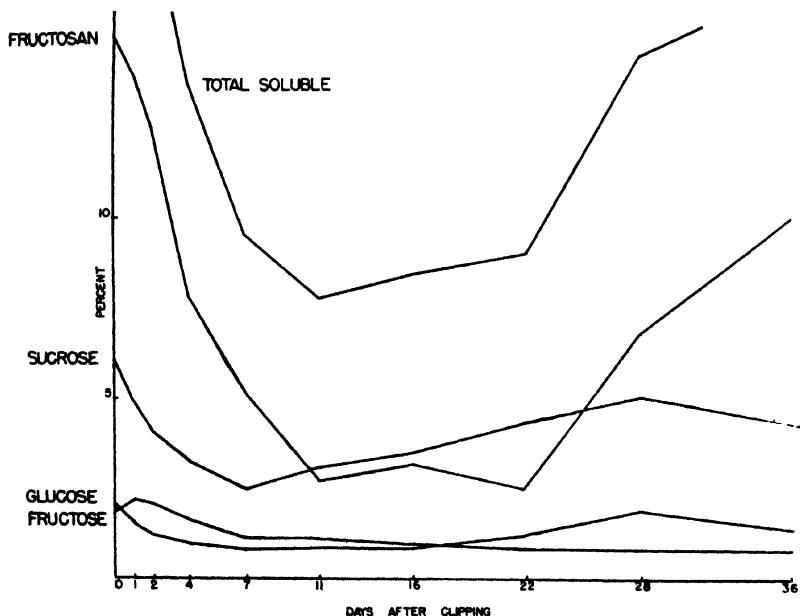
FIG. 2. Water-soluble carbohydrates of the stubble of ryegrass plants growing in the greenhouse for 36 days after removal of the tops.

trated the changes in the water-soluble carbohydrates, fructose, glucose, sucrose, and fructosan and also the total of these constituents in the stubble of plants growing in light from the day of clipping to 36 days thereafter. A rapid decrease in the percentages of all these constituents on the dry weight basis characterized the changes during the first seven days. Since no increase in dry weight had taken place in the stubble or roots during this

time it is assumed that these carbohydrate constituents were being trans-
located to the growing tops and utilized in part as raw material for new
tissue and were also in part being decomposed by respiratory processes to
furnish energy for such growth.   The temporary increase of fructose on the
first day may have been caused by the rapid hydrolysis of fructosan, which
because of its high molecular weight (12) is not translocated as such.   A
minimum content of sucrose was reached about the 7th day and of other
constituents soon thereafter.   A rapid rise in all constituents occurred at
a later date.   The photosynthetic area of new top growth increased suffi-
ciently so that sometime after 11 days of recovery re-storage of carbohy-
urates occurred.   Whether re-storage was in the old tissue or new tissue
could not be determined with the technique used since new as well as old
leaf tissue was included in the stubble zone.

Figure 3 (on the same scale as fig. 2) illustrates similar changes in the
roots.   The concentrations in the roots were smaller, but the relative changes
were as great.
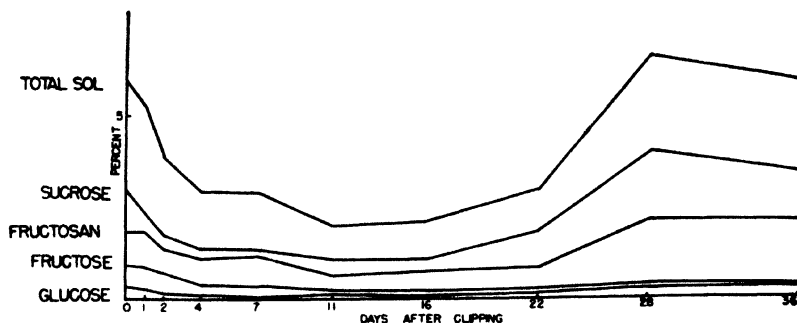
Fig. 3.   Water-soluble carbohydrates of the roots of ryegrass plants growing in the
greenhouse for 36 days after removal of the tops.

Figure 4 presents the changes taking place in the stubble with respect
to the insoluble carbohydrates and lignin.   Total soluble carbohydrates,
represented by the dotted line, is repeated from figure 2 for comparison
purposes.   Cellulose, hydrolyzable pentosan, and lignin percentages did not
show the typical U-shaped curve of the soluble carbohydrates.   The appar-
ent increase of these constituents during the first week may have been a real
increase or may have been partly due to the method of expression in per-
centage of the dry weight.   If soluble carbohydrates alone had been removed
during this period all remaining constituents would have been increased on
a percentage basis.   Actual increase in total cellulose and pentosan may
have occurred since some new tissue was formed.   There was no evidence,
however, of their utilization as in the case of soluble carbohydrates.   After
attaining a maximum at about 11 days there was a slight decrease in cellu-
lose and pentosan.   The basis of expression may again have been the cause
of this since soluble carbohydrates have increased during this later period.·
Lignin did not show the later drop.
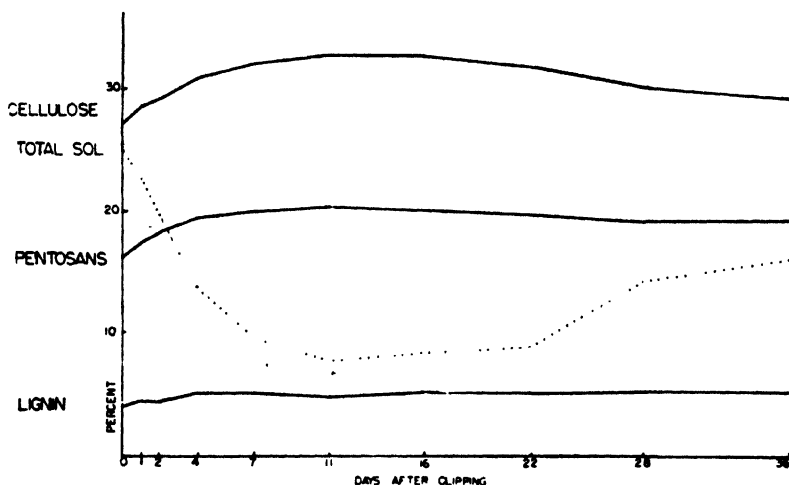
FIG. 4. Cellulose, hydrolyzable pentosans, and lignin (solid lines) and total soluble carbohydrates (dotted line) of stubble of ryegrass plants growing in the greenhouse 36 days after removal of the tops.

Figure 5 furnishes the same information for the roots.   The conclusions to be drawn are similar.

Plants in darkness (figs. 6, 7) showed changes similar to those in the light during the period when top growth was being produced.   Rates of loss of soluble carbohydrates were similar to those of plants in the light except that no re-storage occurred.   All soluble constituents, if illustrated separately, would show similar changes.   Fructosan disappeared entirely by the 22nd day in the stubble and by the 16th day in the roots.   The soluble carbohydrates persisting to the 36th day were, in the stubble, 0.22 per cent. of
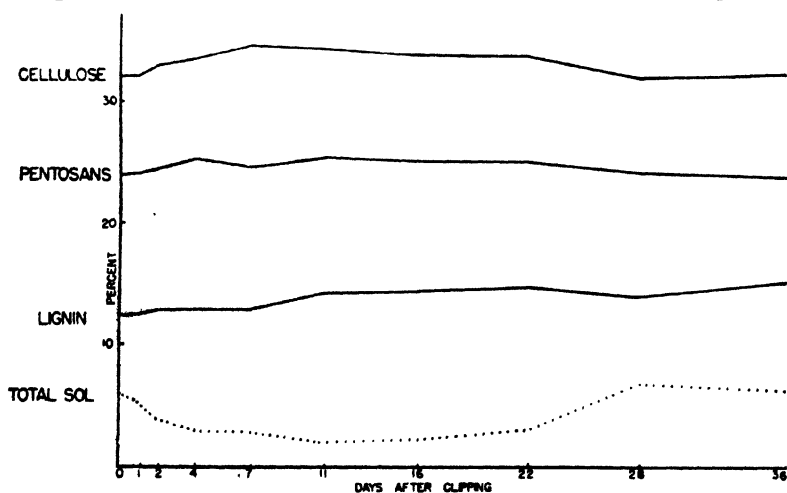
FIG. 5. Cellulose, hydrolyzable pentosans, and lignin (solid lines) and total soluble carbohydrates (dotted line) of roots of ryegrass plants growing in the greenhouse for 36 days after removal of the tops.
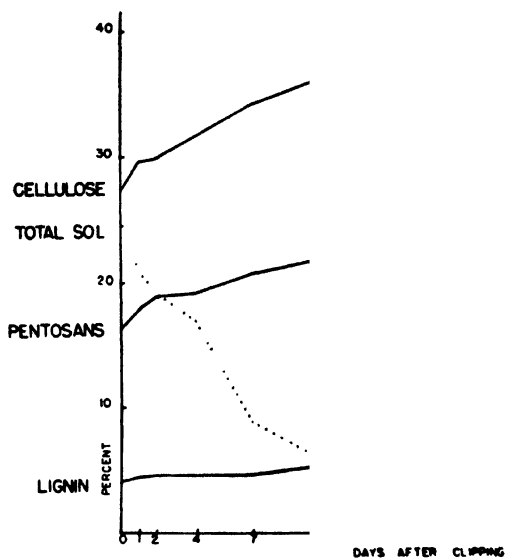
Fig. 6. Cellulose, hydrolyzable pentosans, and lignin (solid lines) and total soluble carbohydrates (dotted line) of stubble of ryegrass plants growing in darkness for 36 days after removal of the tops.

sucrose and 0.41 of glucose; and in the roots, 0.15 per cent. of sucrose and 0.07 of fructose. There was no evidence of utilization of cellulose, pentosan, and lignin.

The changes taking place in several minor carbohydrate constituents are not reported in detail. The oxalic acid hydrolysis liberated reducing sub-
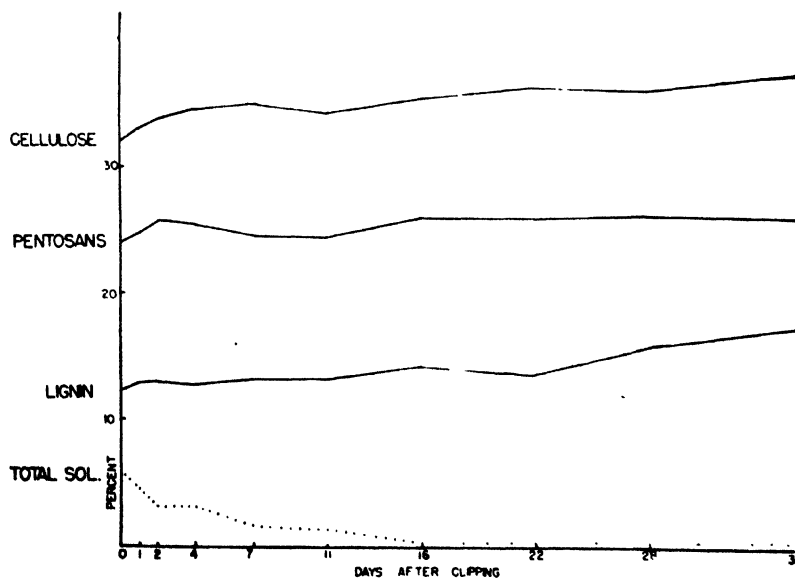
Fig. 7. Cellulose, hydrolyzable pentosans, and lignin (solid lines) and total soluble carbohydrates (dotted line) of roots of ryegrass plants growing in darkness for 36 days after removal of the tops.
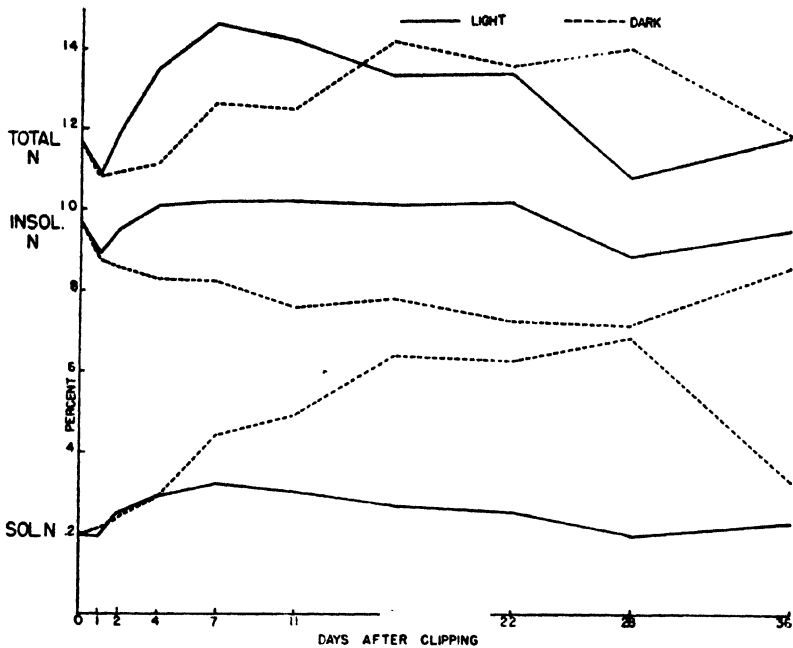
FIG. 8.   Total, insoluble, and soluble nitrogen of stubble of ryegrass plants growing in the greenhouse (solid lines) and in darkness (dotted lines) for 36 days after removal of the tops.
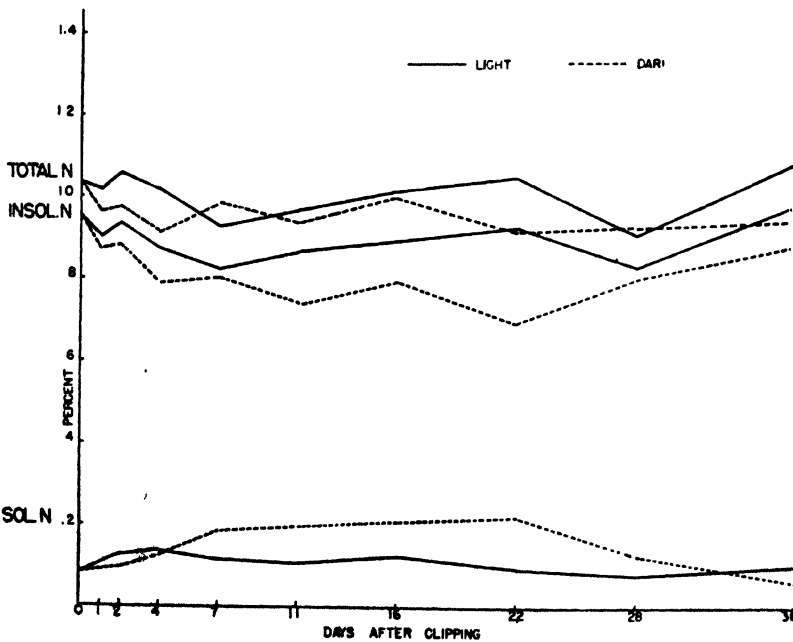
FIG. 9.   Total, insoluble, and soluble nitrogen of roots of ryegrass plants growing in the greenhouse (solid lines) and in darkness (dotted lines) for 36 days after removal of the tops.

stances in addition to fructose which have been calculated as glucose. These substances varied between 0.6 and 1.0 per cent. in the stubble and between 0.4 and 0.6 per cent. in the roots. The sulphuric acid hydrolysis also liberated some fermentable, non-fructose substances which have been calculated as glucose and probably include the portion liberated by oxalic acid. The amounts of these components varied between 2.5 and 4.5 per cent. in the stubble and between 1.0 and 2.0 per cent. in the roots. The uncertainty of the method of calculating sugar mixtures makes it unsafe to attach any importance to these figures.

Soluble, insoluble, and total nitrogen changes in the stubble are illustrated in figure 8. Total nitrogen reached its greatest accumulation in plants in light 7 days after cutting and in plants in the dark somewhat later. The proportion of total nitrogen in soluble form increased from 17 per cent. at cutting time to 22 per cent. at 7 days for plants in the light. In plants in the dark the proportion of soluble nitrogen increased rapidly, amounting to 49 per cent. of the total at 28 days. One fourth of the soluble nitrogen at this date consisted of nitrates which were not detected originally but accumulated rapidly in plants in the dark and later decreased at the time the plants were dying.

Nitrogen changes in the roots are illustrated in figure 9. Total nitrogen showed less fluctuation than did that of the stubble. The amount of nitrogen in the soluble form increased from 8.2 to 13.5 per cent. at four days. In the dark the soluble nitrogen reached 21 per cent. of the total at 11 days; 36 per cent. of this was nitrate.

Discussion

The term "reserve substances" in plants has been freely used. "Reserve carbohydrates" usually are referred to as those carbohydrates which vary in amount and are alternately accumulated and utilized by the plant in its growth cycle or when disturbed by changes in its environment. In some cases carbohydrates occurring in the leafy parts of plants are referred to as reserves; but in pasture plants carbohydrates present in the tops, which are removed by grazing, cannot function as reserves for the plant under grazing conditions.

In this paper no attempt will be made to define "reserves." An attempt is made to describe only changes that take place in the plant parts which remain after partial defoliation. The disappearance or decrease in amount of certain constituents of the roots and stubble during the period when new growth is progressing rapidly, indicates that such substances may be properly called reserves.

The results reported here do not include all carbohydrate changes. For example, the acid-hydrolyzable material, often referred to as hemicellulose, has not been broken down into all of its component parts. The major part of it, excluding fructosan, is believed to be pentosan but it may consist of more than one pentose compound. Some non-pentosan material, minor in

quantity, also occurs. As the results indicate, the total acid-hydrolyzable material does not show any considerable utilization. It is possible that some part of it may have functioned as utilizable material for the synthesis of new compounds but if this were so it was replaced in the synthesis of other materials of similar analytical properties.

Complete exhaustion of any particular compound did not occur with the plants growing in light. Since in this experiment not all of the photosynthetic tissue was removed and since new leaf tissue was being produced throughout the course of the experiment under favorable environmental conditions, synthesis of carbohydrates occurred in sufficient quantity to prevent complete exhaustion of the reserves. The degree of clipping to which the plants were subjected was not particularly severe and the carbohydrate content of the plants was high at the time of cutting. If a second cutting had been made at some period a week or two after the first at a time when soluble carbohydrates were at a much lower level, subsequent recovery of the plants probably would have been greatly retarded. Further work is contemplated along this line.

In plants growing in the dark following clipping, quantitative changes in carbohydrates occurred in a similar manner as in plants growing in the light. This supports the observation of GRABER *et al.* (8) that the growth of plants in darkness might be used to give an indication of the quantity of reserve substances present. When plants were kept for a long period in the dark so that reserves might be considered completely exhausted the constituents removed were the same as those utilized in considerable quantity by plants in light.

Nitrogen reserves have received only minor attention. A nitrate-containing nutrient solution was furnished the plants at all times and the nitrogen content of the plant did not decrease appreciably. There was, however, an increase in the proportion of soluble nitrogen to total nitrogen. This may suggest that proteins are hydrolyzed to more soluble forms. There is little doubt that in plants growing in the dark there was a marked hydrolysis of proteins in the parts studied.

Summary

Changes in the carbohydrate content of roots and stubble of perennial ryegrass grown in gravel cultures in the greenhouse were determined at intervals during a recovery period of 36 days following partial defoliation.

Water-soluble carbohydrates including glucose, fructose, sucrose, and fructosan decreased rapidly for several weeks and then increased. Cellulose, hydrolyzable pentosan, and lignin failed to show any decrease. The soluble carbohydrates alone gave evidence of being reserve substances.

Plants placed in darkness after cutting underwent changes in composition similar to those in light except that soluble carbohydrates continued to decrease almost to the point of exhaustion.

There is evidence that hydrolysis of proteins took place in plants placed in darkness.

LITERATURE CITED

1. Aldous, A. E. Relation of organic food reserves to the growth of some Kansas pasture plants. Jour. Amer. Soc. Agron. **22**: 385–392. 1930.

2. Albert, W. B. Studies on the growth of alfalfa and some perennial grasses. Jour. Amer. Soc. Agron. **19**: 624–654. 1927.

3. Archibold, H. K. Fructosans in the monocotyledons. A review. New Phytol. **39**: 185–219. 1940.

4. Bukey, F. S., and Weaver, J. E. Effects of frequent clipping on the underground food reserves of certain prairie grasses. Ecology **20**: 246–252. 1939.

5. Canfield, R. H. The effect of intensity and frequency of clipping on density and yield of black grama grass and tobosa grass. U. S. Dept. Agr. Tech. Bull. 681. 1939.

6. Crampton, E. W., and Maynard, L. A. The relation of cellulose and lignin content to the nutritive value of animal feeds. Jour. Nutrition **15**: 383–395. 1938.

7. Graber, L. F. Food reserves in relation to other factors limiting the growth of grasses. Plant Physiol. **6**: 43–72. 1931.

8. ——————, Nelson, N. T., Leukel, W. A., and Albert, W. B. Organic food reserves in relation to the growth of alfalfa and other perennial herbaceous plants. Wisconsin Agr. Exp. Sta. Res. Bull. 80. 1927.

9. ——————, and Sprague, V. G. The productivity of alfalfa as related to management. Jour. Amer. Soc. Agron. **30**: 38–54. 1938.

10. Harrison, C. M. Responses of Kentucky bluegrass to variations in temperature, light, cutting and fertilizing. Plant Physiol. **9**: 83–106. 1934.

11. ——————, and Hodgson, C. W. Response of certain perennial grasses to cutting treatments. Jour. Amer. Soc. Agron. **31**: 418–430. 1939.

12. Haworth, W. N., Hirst, E. L., and Lyne, R. R. A water-soluble polysaccharide from barley leaves. Biochem. Jour. **31**: 786–788. 1937.

13. Leukel, W. A. Deposition and utilization of reserve foods in alfalfa plants. Jour. Amer. Soc. Agron. **19**: 596–623. 1927.

14. McCARTY, E. C. Seasonal march of carbohydrates in *Elymus ambiguus* and *Muhlenbergia gracilis,* and their reaction under moderate grazing use. Plant Physiol. **10**: 727–738. 1935.

15. ——————. The relation of growth to the varying carbohydrate content in mountain brome. U. S. Dept. Agr. Tech. Bull. 598. 1938.

16. ——————, and PRICE, R. Growth and carbohydrate content of important mountain forage plants in central Utah as affected by clipping and grazing. U. S. Dept. Agr. Tech. Bull. 818. 1942.

17. MOROZOV, A. S. Storing of carbohydrates by forage grasses. Comp. Rend. Acad. Sci. U. R. S. S. **24**: 407–409. 1939.

18. NORMAN, A. G. The composition of forage crops. I. Ryegrass (Western Wolths). Biochem. Jour. **30**: 1354–1362. 1936.

19. ——————, and RICHARDSON, H. L. The composition of forage crops. II. Ryegrass (Western Wolths). Changes in herbage and soil during growth. Biochem. Jour. **31**: 1556–1566. 1937.

20. ——————, WILSIE, C. P., and GAESSLER, W. G. The fructosan content of some grasses adapted to Iowa. Iowa State College Jour. Sci. **15**: 301–305. 1941.

21. PARKER, K. W., and SAMPSON, A. W. Growth and yield of certain Gramineae as influenced by reduction of photosynthetic tissue. Hilgardia **5**: 361–381. 1931.

22. PHILLIPS, T. G. Semimicromethod for determining copper reduced by sugars. Jour. Assoc. Off. Agr. Chem. **24**: 181–182. 1941.

23. RICHARDSON, A. E. V., TRUMBLE, H. C., and SHAPTER, R. E. The influence of growth stage and frequency of cutting on the yield and composition of perennial grass, *Phalaris tuberosa.* Australian Council Sci. Ind. Res. Bull. 66. 1932.

24. ROBERTSON, J. H. Effect of frequent clipping on the development of certain grass seedlings. Plant Physiol. **8**: 425–447. 1933.

25. SAMPSON, A. W., and McCARTY, E. C. Carbohydrate metabolism of *Stipa pulchra.* Hilgardia **5**: 61–100. 1930.

26. STAPLEDON, R. G., and MILTON, W. E. J. The effect of different cutting and manurial treatments on the tiller and root development of cocksfoot. Welsh Jour. Agr. **6**: 166–174. 1930.

27. WEINMANN, H. Storage of root reserves in Rhodes grass. Plant Physiol. **15**: 467–484. 1940.

28. ——————. Seasonal chemical changes in the roots of some South African highveld grasses. Jour. South African Bot. **6**: 131–145. 1940.

29. WILLARD, C. J. Root reserves of alfalfa with special reference to time of cutting and yield. Jour. Amer. Soc. Agron. **22**: 595–602. 1930.

EFFECT OF OXYGEN CONCENTRATION ON THE RESPIRATION OF SOME VEGETABLES[1]

HANS PLATENIUS

(WITH SEVEN FIGURES)

Introduction

Commercial methods of storing fruits and vegetables in modified atmosphere are based on the fact that respiration, ripening, and other physiological processes can be retarded by maintaining an atmosphere in which the oxygen content is lower and the carbon dioxide concentration higher than in normal air. Many investigators assume that it is the presence of carbon dioxide rather than the limited oxygen supply which has a depressing effect on the physiological activity of the plant tissue. In fact, modified atmosphere storage is frequently spoken of as "carbon dioxide storage."

There is reason to believe, however, that the importance of a limited oxygen supply itself has been underestimated. Indirect evidence for this view is found in the results of THORNTON (8) which make it clear that the presence of carbon dioxide in the storage atmosphere does not always depress the respiration of plant material. On the contrary, he found that the respiration rate of potatoes and onions was markedly increased when held in an atmosphere of normal oxygen content to which varying quantities of carbon dioxide had been added. On the other hand, the same treatment had a depressing effect on the respiratory activity of asparagus and strawberries.

In the literature few experiments are reported which deal exclusively with the effect of low concentrations of oxygen on the respiration of plant tissue. Some of these experiments were conducted for a few hours only, and there is no assurance that the results would have been the same had the storage period been extended to several days. Other studies were carried out in the complete absence of oxygen. Obviously, the course of respiration under anaerobic conditions is entirely different from that which is followed when a low, but constant supply of oxygen is maintained.

Most textbooks merely state that the oxygen concentration of the air can be changed over a wide range without materially affecting the rate of respiration. Evidence cited in a review of the literature by MACK (5) indicates that such a broad statement is subject to several exceptions. PARIJA (6) observed that the production of carbon dioxide of apples was lowest when the oxygen concentration of the air was about 5 per cent.; it rose to higher levels above and below this critical concentration. STEWARD, BERRY, and BROYER (8), working with thin disks of carrot and artichoke tissue suspended in weak salt solutions, observed that aeration of the solution with a gas mixture containing only 2.7 per cent. oxygen markedly depressed the carbon dioxide production of the tissues. On the other hand, raising the

oxygen concentration of the aeration stream to 40 per cent. did not result in respiration rates higher than were observed in normal air. More recently CHOUDHURY (3) investigated the effect of different oxygen concentrations on the respiration of several vegetables. He found that the respiration rate of potatoes remained fairly constant at oxygen concentrations ranging from 6.2 to 98.6 per cent. Artichokes decreased in respiratory intensity at oxygen levels below normal, and the respiration rate of carrots increased or decreased with corresponding changes in the supply of oxygen.

What little experimental evidence is available points to the conclusion that the effect of low oxygen concentrations on respiration varies with different kinds of plant tissue. This is to be expected considering the wide variations in the normal rate of respiration and the differences in relative surface and permeability to gases of plant material. Moreover, the results are likely to be influenced by the length of exposure, the temperature, and other experimental conditions.

The following experiments were conducted for the purpose of obtaining data which would serve as a basis for developing practical methods of modified atmosphere storage. It is reasonable to expect that any combination of gases in the storage atmosphere which effectively reduces the normal respiration rate also retards the process of deterioration. Carbon dioxide gas in the respiration chamber was kept at a fraction of one per cent. in order to study respiration at low levels of oxygen without the complicating effect of another variable. Oxygen consumption as well as carbon dioxide production were measured because both gases play an equally important rôle in the process of respiration. In particular, it was desired to know how far the oxygen concentration could be reduced without affecting the normal course of respiration.

Methods

All experiments were carried out in constant temperature rooms where fluctuations in temperature did not exceed 2° C. Except for brief periods when readings were taken, the vegetables were kept in the dark. Oxygen consumption and carbon dioxide production were measured simultaneously by a method described in detail in an earlier paper (7). Carbon dioxide was absorbed by a normal solution of sodium hydroxide in the bottom of the chamber while oxygen entered the chamber under atmospheric pressure at the same rate as it was utilized by the respiring tissue. At the beginning of each experiment, the chamber was flushed with nitrogen long enough to reduce its oxygen content approximately to the level desired. The exact oxygen concentration was determined by gas analysis at the beginning and end of each experiment. In all instances, the original gas composition was maintained within 0.3 per cent. throughout the experiment.

Readings of oxygen consumption and carbon dioxide production were taken at intervals of 24 hours. All data were expressed on the basis of the original fresh weight. Because of the high humidity in the chambers, there

were practically no losses from transpiration and total weight losses over a period of several days were less than 5 per cent.

Methods approved by the Association of Official Agricultural Chemists (1) were used for chemical analyses. Sugars were determined by weighing the cuprous oxide precipitate. The quantity of soluble nitrogen was found by evaporating the alcoholic extract under vacuum, treating the residue with sodium sulphite, and digesting it with a mixture of sulphuric acid, salicylic acid, and mercuric oxide. Protein nitrogen was determined in the residue from the alcohol extraction, using the Kjeldahl-Gunning-Arnold method.

Vegetables used for these experiments were garden-fresh, harvested less than two hours before each experiment was started. Great care was taken to have comparable samples for storage under different oxygen concentrations. Each lot was divided into smaller ones containing an identical number of individuals and these were as uniform in size and shape as could be obtained by careful selection. According to the size of individual specimens, samples ranging from 100 to 700 grams were used in the respiration studies. In no instance did a sample contain less than 15 specimens.

Results

ASPARAGUS

In the first series of experiments, the gas exchange of asparagus held at a temperature of 20° C. was studied simultaneously at nine different levels of oxygen ranging from 1.0 to 20.5 per cent. In figure 1 the average rates
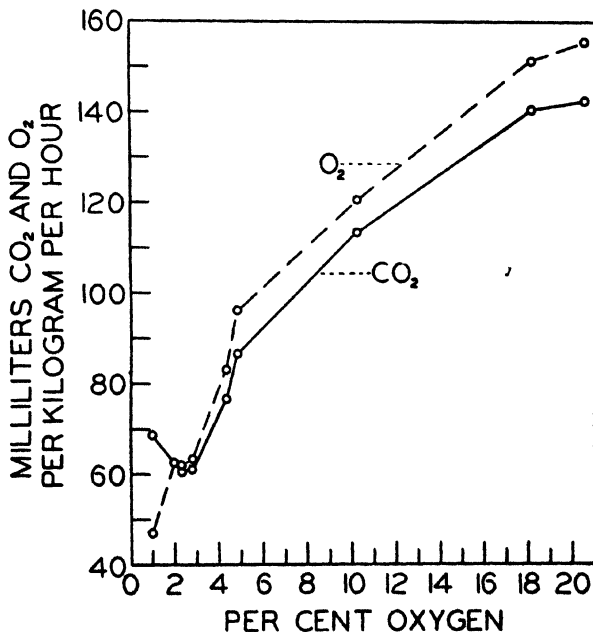
FIG. 1. Average rates of oxygen consumption and carbon dioxide production of asparagus at nine levels of atmospheric oxygen during a three-day period.

of carbon dioxide evolution and oxygen consumption during a three-day
interval are plotted against the oxygen concentrations maintained in the
different chambers.   Attention is called to the fact that the abscissa repre-
sents oxygen concentrations, not time intervals.   On the ordinate, respiration
rates are plotted in terms of milliliters.   Using this expression instead of
milligrams, deviations from unity in the respiratory quotient can be recog-
nized at a glance by inspecting the relative position of the two curves.

The data show that any change in the oxygen content of the storage
atmosphere caused corresponding changes in the respiration rate of the
tissue.   This effect became increasingly pronounced as lower levels of oxy-
gen were approached.   Based on the values for carbon dioxide production,
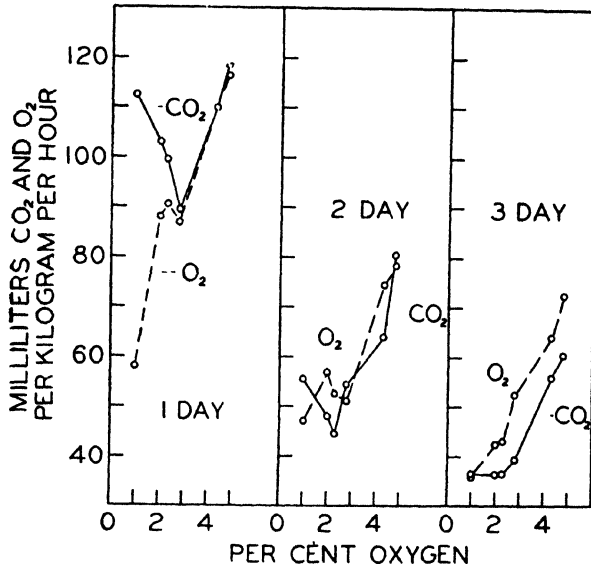
Fig. 2.   Average daily rates of oxygen consumption and carbon dioxide production
of asparagus at low levels of atmospheric oxygen.

a minimum in respiratory activity was reached at an oxygen concentration
of 2.3 per cent.   At this point the respiration rate was only 40 per cent.
of that occurring in normal air.   At still lower levels, carbon dioxide pro-
duction began to rise again while oxygen consumption continued to fall off
sharply.   According to the terminology used by BLACKMAN (2), 2.3 per
cent. of oxygen represents the "extinction point of N R"; it is the concen-
tration at which the type of respiration occurring in pure nitrogen becomes
extinct.   Above the extinction point of N R sufficient oxygen is available
to maintain aerobic respiration and to suppress fermentation completely.
Below this point aerobic respiration still takes place but diminishes rapidly
in intensity.   Fermentation, on the other hand, increases; at a concentra-
tion of one per cent. oxygen, the combined output of carbon dioxide from
aerobic and anaerobic respiration becomes considerably larger than it is at
the extinction point.

In figure 2 the respiration rates for the range from 1.0 to 4.8 per cent. of oxygen are plotted separately for each of the three days. Following the usual drift that occurs with time, the respiratory activity declined at any one oxygen concentration during successive days. It is also evident that the position of the extinction point shifted with time. During the first day, it was located at 2.8 per cent.; during the second, at 2.3 per cent.; and during the third day it had dropped to 1.0 per cent. This shift of the extinction point is probably associated with the normal decline in respiratory intensity as time progresses. At any rate, it shows that the tissue becomes more tolerant to low oxygen concentrations as the storage period is lengthened.

FIG. 3. Injury to asparagus stalks caused by anaerobic respiration at concentrations of atmospheric oxygen below 2.8 per cent.

There was evidence that anaerobic respiration at low levels of oxygen resulted in the accumulation of toxic end products of incomplete oxidation. This was indicated by definite signs of injury of the tissue which appeared after one day of storage in all the samples held at oxygen concentrations of less than 2.8 per cent. As shown in figure 3, sections of the external tissue had collapsed, forming deep longitudinal channels in the upper portion of the spears. These symptoms of injury were identical with those that are produced when asparagus is covered with a fairly thick coat of wax or when spears are exposed to temperatures just above the freezing point for several weeks. Aside from visible injury, the samples held at one and two per cent. oxygen had a pronounced musty and alcoholic odor at the time the experiment was discontinued. It is possible that the injury to the tissue caused

changes in the permeability to gases. This would explain the secondary rise in oxygen consumption in the range between 2.0 and 2.8 per cent. oxygen concentration which was observed during the first and second day of storage. The sharp rise in carbon dioxide production with decreasing levels of oxygen likewise may be attributed to increased permeability of the tissue, although the principal reason for this rise is to be sought in conditions becoming more favorable for rapid anaerobic respiration.

The practical significance of reduced respiration at low levels of oxygen is that losses in carbohydrates, which in asparagus consist chiefly of sugars, are greatly reduced. This conclusion is substantiated by the results of chemical analyses taken at the beginning and end of the storage period. Table I shows that after three days of storage in normal air, asparagus had

TABLE I

EFFECT OF OXYGEN CONCENTRATION IN THE ATMOSPHERE ON TOTAL SUGARS, PROTEIN AND SOLUBLE NITROGEN OF ASPARAGUS HELD AT 20° C. DATA ARE EXPRESSED ON THE BASIS OF ORIGINAL FRESH WEIGHT

OXYGEN CONTENT OF THE STORAGE ATMOSPHERE	STORAGE PERIOD	TOTAL SUGARS	SUGARS RETAINED*	PROTEIN NITROGEN	SOLUBLE NITROGEN
%	days	%	%	%	%
	0	2.32	100.0	0.315	0.093
20.5	3	1.39	59.9	0.244	0.129
10.2	3	1.56	67.2	0.249	0.146
4.8	3	2.09	90.1	0.260	0.149
2.8	3	2.13	91.8	0.283	0.125
2.0	3	2.01	86.6	0.253	0.154

* Percentage of original.

lost 40 per cent. of its original sugar content whereas only eight per cent. had been lost at an oxygen concentration of 2.8 per cent. In other words, sugar losses from asparagus under low oxygen were only one-fifth of those occurring in normal air.

Comparing the respiration data with the results of the chemical analyses, it became apparent that in any one treatment sugar losses account for only one-half to two-thirds of the total quantity of carbon dioxide evolved. The same observation was made by the writer in earlier experiments (7), and at that time the suggestion was offered that part of the substrate used in respiration of asparagus is furnished by the products of protein hydrolysis. The present data confirm this assumption. There was a definite decrease in protein nitrogen with a corresponding rise in soluble nitrogen during storage. Proteolysis was most active in the sample stored in normal air, it was somewhat slower at low concentrations of oxygen.

Essentially the same trends in respiration rates were observed when asparagus was stored at a temperature of 10° C. at three levels of oxygen. Because of technical difficulties, records were obtained only for carbon dioxide production. The results of this experiment (table II) show that

TABLE II

RESPIRATION RATES OF ASPARAGUS STORED AT 10° C. AND AT THREE LEVELS
OF OXYGEN FOR SEVEN DAYS

OXYGEN CONTENT OF THE STORAGE ATMOSPHERE	MILLILITERS CARBON DIOXIDE PER KILOGRAM PER HOUR					
	1ST DAY	2ND DAY	3RD DAY	4TH AND 5TH DAY	6TH AND 7TH DAY	AVERAGE FOR SEVEN-DAY PERIOD
%	ml.	ml.	ml.	ml.	ml.	ml.
20.5	86.3	68.9	61.9	52.8	53.6	61.4
2.1	57.5	40.8	51.9	41.7	28.5	41.5
1.2	44.2	28.3	28.6	20.3	25.2	27.4

the respiration rate could be reduced as much as 55 per cent. by lowering
the oxygen content of the storage atmosphere to 1.2 per cent. Significant
is the fact that 1.2 per cent. oxygen in the air was sufficient to maintain
aerobic respiration at a temperature of 10° C. After seven days, the sam-
ples showed no signs of injury nor was there any odor of alcohol perceptible
in the chambers. Also, the fact that the carbon dioxide production at 1.2
per cent. oxygen was considerably lower than it was at 2.1 per cent. indi-
cates that at a temperature of 10° C. the extinction point of N R lies close
to 1.2 per cent. oxygen or even lower.

SPINACH

The respiration rates of spinach held for three days at a temperature
of 20° C. were measured at three levels of oxygen. The results are ex-
pressed graphically in figure 4. Obviously, the number of points definitely
located is too small to show more than a general trend in the slope of the
curves. It is apparent, however, that changes in the oxygen concentration
above five per cent. had little effect on carbon dioxide production. Only at
an oxygen level of 0.8 per cent. was a significant reduction in respiration
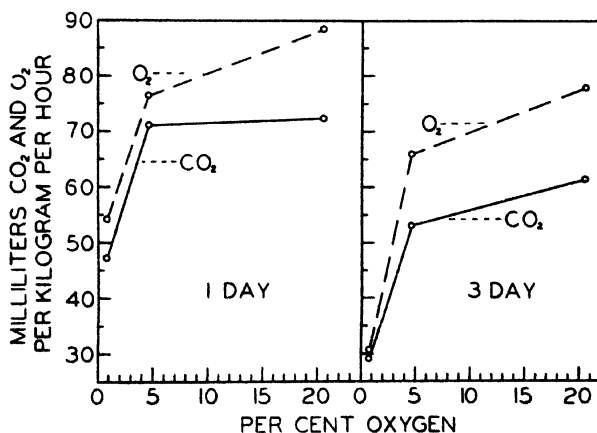
FIG. 4. Rates of oxygen consumption and carbon dioxide production of spinach at
three levels of oxygen during the first and third day of storage.

rate obtained.   At that concentration, carbon dioxide output had decreased to 65 per cent. on the first, and to 48 per cent. on the third day of storage. In no instance did carbon dioxide production exceed oxygen consumption, although the respiratory quotient showed a tendency to become larger at lower levels of oxygen.

Spinach in normal air has an unusually low respiratory quotient.   This may be due to the continuous formation of oxalic acid or other organic compounds of high oxygen content or it may be caused by the decomposition of proteins and their utilization as substrate in respiration.   Whatever the reaction involved may be, a lowering of the oxygen content of the storage atmosphere seems to inhibit that process.   It is of practical significance that an oxygen concentration of 0.8 per cent. reduced respiration to nearly one-half its normal rate, but was sufficient to maintain normal aerobic respiration.   The sample held in that particular gas mixture showed no signs of injury; in fact, it was definitely superior in appearance and taste to that stored in normal air.

SNAP BEANS

As shown in figure 5, the response of snap beans to low concentrations of
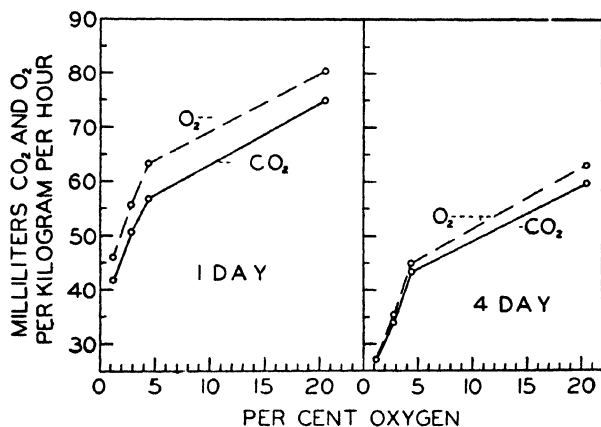
FIG. 5.   Rates of oxygen consumption and carbon dioxide production of snap beans at four levels of atmospheric oxygen during the first and fourth day of storage.

oxygen at a temperature of 20° C. was similar to that of spinach.   Reducing the oxygen content of the air to 4.4 per cent. had little effect; but at a concentration of 1.2 per cent. the average carbon dioxide evolution during the four-day period was only about 50 per cent. of that which was produced in normal air.   Within the experimental range of oxygen concentrations, the respiratory quotient always remained below unity, and this is in accord with the fact that none of the samples developed symptoms of injury which could be traced to anaerobic respiration.

SHELLED PEAS

The respiration curves for shelled peas at a temperature of 20° C. differed in several respects from those of other vegetables.   Figure 6 shows
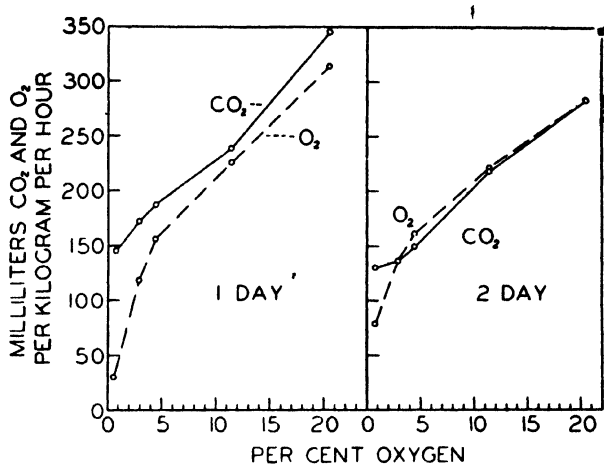
FIG. 6. Rates of oxygen consumption and carbon dioxide production of shelled peas at five levels of oxygen during the first and second day of storage.

that within the experimental range a nearly linear relationship existed between carbon dioxide production and the oxygen content of the air. The respiratory quotient was well above 1.0 at all concentrations during the first day but dropped to a level close to unity a day later. Only at the lowest oxygen level did carbon dioxide production exceed oxygen consumption consistently.

Fermentation occurred in the samples held at oxygen concentrations of 0.8 and 2.8 per cent. This was evident from the strong alcoholic odor of these particular samples and from the pronounced off-flavor before and after cooking. Moreover, the surface of these peas showed a brown discoloration, and the seed coat of some had burst. Although there can be no doubt that in these samples fermentation occurred simultaneously with aerobic respiration, the combined production of carbon dioxide from these two processes did not cause a secondary rise in carbon dioxide output at extremely low oxygen levels as had been observed in asparagus. It is almost impossible, therefore,

TABLE III

EFFECT OF OXYGEN CONCENTRATION IN THE ATMOSPHERE ON TOTAL SUGARS OF SHELLED PEAS HELD AT 20° C. DATA ARE EXPRESSED ON THE BASIS OF ORIGINAL FRESH WEIGHT

OXYGEN CONTENT OF THE STORAGE ATMOSPHERE	STORAGE PERIOD	TOTAL SUGARS	SUGAR RETAINED*
%	days	%	%
	0	6.43	100.0
20.5	2	1.76	27.4
11.4	2	3.09	48.1
4.4	2	4.46	69.4
2.9	2	4.44	69.1

' Percentage of original.

to establish the exact location of the extinction point of N R from inspection of the respiration curves alone.

In commercial practice beneficial results can be expected from storing peas under low oxygen tension, provided the concentration remains sufficiently high to prevent fermentation. At a temperature of 20° C., the best results were obtained at an oxygen level of 4.4 per cent. At this concentration, the average carbon dioxide production was reduced 56 per cent. The degree to which sugar losses were inhibited was even more pronounced than the respiration data would indicate. As shown in table III, the control lot had retained only 27 per cent. of its original sugar content while 69 per cent., that is, more than 2½ times as much, was retained in the sample kept at 4.4 per cent. oxygen.

CARROTS

The respiratory behavior of carrot roots, illustrated in figure 7, changed considerably during the six-day storage period. The maximum reduction
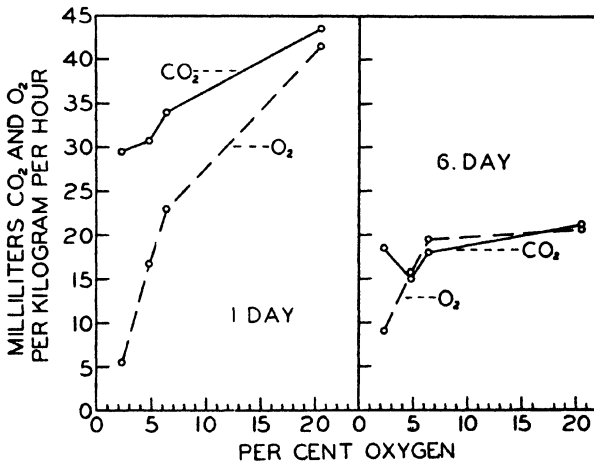
FIG. 7. Rates of oxygen consumption and carbon dioxide production of carrots at four levels of oxygen during the first and sixth day of storage.

in carbon dioxide production observed within the experimental range remained close to 30 per cent. regardless of the fact that the respiratory intensity of all samples declined steadily as time progressed. In the beginning, the respiratory quotient ranged from 1.05 in normal air to 5.8 at 2.3 per cent. oxygen. Gradually the respiratory quotient became smaller, and on the sixth day, two of the samples had reached values slightly below unity. Only at the lowest oxygen concentration did the quotient remain above 2.0 at all times. After the first day, a secondary rise in carbon dioxide production was observed at oxygen levels below 4.8 per cent. This rise persisted; in fact, it became more pronounced during the latter part of the storage period.

Only the sample held at an oxygen concentration of 2.3 per cent. appeared to be permanently injured by toxic end-products of anaerobic respi-

ration. This particular sample had lost its turgor and showed all the other symptoms of injury from an insufficient oxygen supply. The carrots kept at an oxygen level of 4.8 per cent., the next higher concentration employed, remained in sound condition in spite of the fact that the initial respiratory quotient was as high as 1.8.

In this connection, it should be mentioned that in another experiment carrots were kept in excellent condition for six months when stored at a temperature of 2° C. in an atmosphere containing one to two per cent. oxygen. This serves as additional proof that the danger of injury from anaerobic respiration is lessened as the storage temperature is lowered.

Discussion

In analyzing the results of respiration experiments, one must keep in mind that the measured rate of gas exchange deals only with two compounds of the entire chain of reactions that make up the complex process of respiration. Without quantitative chemical analyses showing which organic constituents serve as substrates and which compounds are formed as intermediate and end products, it is impossible to gain a clear understanding of the mechanism of plant respiration. Even if data of such a detailed study were available for one kind of plant tissue, it would be a fallacy to assume that different species or different organs of the same plant have the same respiratory mechanism. Moreover, there is sufficient evidence to show that the course of respiration of any one tissue may change materially with variations in temperature and other environmental conditions. Interpretations of the experimental data of this study are made with these limitations in mind.

An examination of the data on oxygen absorption shows that within the experimental range any decrease in the concentration of atmospheric oxygen produced a corresponding drop in the rate at which oxygen was consumed. This relationship was by no means a linear one. An increment in the oxygen content of the air caused a greater increase in oxygen consumption at low levels of oxygen than it did at levels close to normal. Also, the response to changes in oxygen concentration was more pronounced in the beginning of the storage period than later. Finally, the magnitude of the response varied with different types of tissue.

These observations lead to the conclusion that in the five vegetables examined, oxygen becomes a limiting factor of respiration whenever its concentration drops below that of normal air; and the extent to which the utilization of oxygen is reduced depends on the partial pressure of the gas in the air, the relative respiratory intensity in normal air, and possibly to the permeability of the tissue to oxygen.

The data for carbon dioxide production can be interpreted correctly only if the corresponding values of oxygen are taken into account. The rôle of oxygen in respiration is two-fold: It is a reactant in normal aerobic respiration, and it inhibits anaerobic anabolism. The second function of oxygen is

known as the PASTEUR effect. As defined by DIXON (4) it is the action of
oxygen on living cells which reduces carbohydrate destruction and sup-
presses or diminishes the accumulation of the products of anaerobic metabo-
lism. The PASTEUR effect comes into play whenever the storage atmosphere
contains small quantities of oxygen but complete depression of anaerobic
respiration is not attained until a certain threshold concentration is reached.
BLACKMAN (2) has called this threshold concentration the extinction point
of N R, that is the concentration of oxygen at which the course of respira-
tion followed in an atmosphere of pure nitrogen ceases to exist. Below the
extinction point, carbon dioxide production, as measured experimentally,
consists of the combined gaseous end products of aerobic and anaerobic
respiration. Depending on the intensity of anaerobic respiration, the values
for total carbon dioxide output may actually increase below the extinction
point. This was found to be true in asparagus during the first and second
day and in carrots after the first day of storage. A similar secondary rise
below the extinction point was observed by BLACKMAN (2) in apples held at
low concentrations of oxygen. In these tissues the extinction point of N R
is obviously close to the oxygen concentration at which total carbon dioxide
production is at a minimum.

It is more difficult to determine the position of the extinction point in
plant tissues which fail to show a secondary rise in carbon dioxide produc-
tion. A sudden increase in the respiratory quotient may indicate the be-
ginning of anaerobic respiration, but it must be remembered that a quotient
above unity in itself cannot be taken as evidence that fermentation is taking
place. The respiratory curves of shelled peas, for instance, show a respira-
tory quotient considerably higher than unity at all concentrations within
the experimental range; but injury from anaerobic respiration was apparent
only in the samples held at oxygen levels of less than three per cent. It is
evident, therefore, that the exact location of the extinction point cannot al-
ways be determined by inspection of the respiration data. In fact, for prac-
tical purposes the appearance of symptoms of injury and the presence of an
alcoholic odor in the chamber seem to be a safer and more sensitive index of
how far the oxygen content of the storage atmosphere can be lowered without
harmful effects.

Important is the fact that different plant tissues vary considerably in
their tolerance to low levels of oxygen in the atmosphere. Oxygen concen-
trations of less than one per cent. were sufficient to maintain normal aerobic
respiration in spinach and snap beans while shelled peas did not tolerate
levels below four per cent. Also, there was evidence that the tolerance to
low oxygen increases with the aging of the tissue or with a lowering of the
storage temperature.

The degree to which the rate of respiration could be reduced varied with
different vegetables. In some, the reduction exceeded fifty per cent.; in
others it was barely forty per cent. for the entire storage period. Certainly,
these results conflict with the statement commonly made that the oxygen

content of the air has little influence on the respiratory rate of plant tissue. In fact, the magnitude of the effect of low oxygen storage on respiration suggests that the benefit from modified atmosphere storage, in which part of the oxygen is replaced by carbon dioxide, is due chiefly to the limited oxygen supply rather than to the presence of carbon dioxide. Additional experimental data are needed to determine quantitatively the effect that these two gases have on the respiration rate of various fruits and vegetables.

On the basis of results obtained thus far, it appears that holding fruits and vegetables in an atmosphere of low oxygen content offers possibilities as a practical method of storage. It has been shown that in some vegetables the retention of sugars is even greater than could be expected from the respiration data. Moreover, recent unpublished data have demonstrated that low oxygen is equally effective in retarding the destruction of ascorbic acid.

Several limitations and technical difficulties must be recognized. Aside from the fact that low oxygen storage requires gas-tight storage rooms, provisions have to be made for the continuous removal of carbon dioxide. In order to avoid injury from anaerobic respiration and to obtain maximum benefits, the oxygen concentration in the atmosphere must be maintained within a narrow range, the exact range being specific for each crop and each temperature. It also should be pointed out that many fruits and vegetables spoil because of storage diseases long before an appreciable loss in carbohydrates has occurred. In the course of recent experiments, it was frequently observed that molds and bacteria grow equally well at an oxygen concentration of one per cent. as in normal air. Even the presence of 20 per cent. carbon dioxide in the atmosphere was ineffective in controlling the spread of storage diseases. Consequently, beneficial results from low oxygen storage can be expected only for those crops which are highly perishable because rapid respiration or oxidation of ascorbic acid are important factors in deterioration.

Summary

Five vegetables, asparagus, spinach, snap beans, shelled peas, and carrots were held in respiration chambers in which part of the oxygen was replaced by nitrogen. A definite concentration of oxygen within the range of 0.8 to 20.5 per cent. was maintained in each chamber. Average daily rates of oxygen consumption and carbon dioxide production were determined.

The critical oxygen concentration below which the tissue was injured by anaerobic respiration (extinction point of N R) was about one per cent. for spinach and snap beans, 2.5 per cent. for asparagus, and four per cent. for peas and carrots when held for several days at 20° C. There was evidence that the tissue became more tolerant to low oxygen with aging and with a lowering of the storage temperature.

By choosing the most effective oxygen concentration, the respiration rate as measured by carbon dioxide production could be reduced about 50 per

cent. At the most effective oxygen level, the respiration rate of asparagus was 40, and that of carrots 65, per cent. of the rate in normal air.

Sugar losses occurring in the course of respiration were determined for asparagus and peas. At the most effective levels of oxygen, asparagus retained eight times and peas two and one-half times as much sugar as comparable samples in normal air. It was shown that in asparagus proteins furnish about one-third of the substrate of respiration.

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LITERATURE CITED

1. ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS. Official and tentative methods of analysis. Washington, D. C. 1935.

2. BLACKMAN, F. F. Analytical studies in plant respiration. III. Formulation of a catalytic system for the respiration of apples and its relation to oxygen. Proc. Roy. Soc. (London) 103B: 491–523. 1928.

3. CHOUDHURY, J. K. Researches on plant respiration. V. On the respiration of some storage organs in different oxygen concentrations. Proc. Roy. Soc. (London) 127B: 238–257. 1939.

4. DIXON, KENDAL CARTWRIGHT. The Pasteur effect and its mechanism. Biol. Rev. 12: 431–460. 1937.

5. MACK, W. B. The relation of temperature and the partial pressure of oxygen to respiration and growth in germinating wheat. Plant Physiol. 5: 1–68. 1930.

6. PARIJA, P. Analytical studies in plant respiration. II. The respiration of apples in nitrogen and its relation to respiration in air. Proc. Roy. Soc. (London) 103B: 446–490. 1928.

7. PLATENIUS, HANS. Effect of temperature on the respiration rate and the respiratory quotient of some vegetables. Plant Physiol. 17: 179–197. 1942.

8. STEWARD, F. C., BERRY, W. E., and BROYER, T. C. The absorption and accumulation of solutes by living plant cells. VIII. The effect of oxygen upon respiration and salt accumulation. Ann. Bot. 50: 345–366. 1936.

9. THORNTON, NORWOOD C. Carbon dioxide storage. III. The influence of carbon dioxide on the oxygen uptake by fruits and vegetables. Contrib. Boyce Thompson Inst. 5: 371–402. 1933.

GERMINATION, GROWTH, AND RESPIRATION OF RICE AND BARLEY SEEDLINGS AT LOW OXYGEN PRESSURES

J. Vlamis and A. R. Davis

(WITH FIVE FIGURES)

An extreme sensitivity to oxygen deficiency is demonstrated by barley seeds undergoing germination. The presence of a film of water above the seeds, for example, inhibits their germination and rapidly leads to fermentation of a pronounced character with a rapid disintegration of the tissues. This mortality may be partly reduced by forcing a stream of air through the water.

On the other hand, it is a common agricultural practice to germinate rice seeds in standing water 1 to several centimeters in depth. This behavior of rice has been observed on several occasions but with one recent exception, no attempt has been made to establish the oxygen relations of germinating rice on a quantitative basis.

In an exhaustive review of the literature on oxygen relations of plants (6), the general effects on the respiration of a wide variety of plants show an independence of oxygen until a partial pressure of 2 per cent. oxygen is reached. Below this point a very sharp decline follows in respiration, measured in most experiments by carbon dioxide production.

Mack (6) germinated wheat seeds aerobically and after 42 hours transferred the seedlings to 12 different oxygen tensions ranging from 0.6 per cent. to 98.3 per cent. and covering five temperatures from 10° C. to 30° C. at 5 degree intervals. The carbon dioxide evolved was measured at several intervals within a 46-hour period and the shoot lengths determined at the end of this time. The anticipated inter-dependence of temperature and oxygen was found. The respiration curves for each of the 3 highest temperatures, oxygen taken as the variable, assume parallel shapes differing only as to absolute values. The effect on shoot growth is greater than that on respiration in response to temperature change, and to oxygen at the lowest pressures. The maximum in respiration occurs uniformly at 30° C., and the shoot growth attains a maximum between 20° and 25° C.

Takahashi (11) germinated rice in oxygen-free water but growth was limited to a small amount of plumule development which ceased after reaching a length of 3 cm. Yokoi (14) likewise obtained only shoot growth of rice under water, and in sand cultures the relative growth of the radicle increased at the expense of the shoot as the water content of the sand declined. These observations have been repeated by Nagai (10). The germination of rice on the surface of submerged soil has been found to be superior to that of seeds placed below the soil (5).

The catalase activity in barley, wheat, and rice has been compared in aerobic and anaerobic atmospheres (7). The catalase activity was shown to

decrease in aerobically germinated seeds. Bermuda grass and cat-tail have been found to have a superior germination under reduced oxygen pressure (**8, 9**).

Respiratory quotients of barley and several types of rice seeds germinated in air and water have been found to vary considerably (**1**), but the existing oxygen pressures in the environment were not determined.

A recent comparison of wheat and rice showed a rapid decline in growth and in oxygen consumption for both types of seeds as the partial pressure of oxygen was lowered (**12**). The total carbon dioxide production likewise declined for wheat but increased with rice and reached a maximum as oxygen pressure approached zero. . These results were obtained on seedlings germinated aerobically for brief periods and placed under the experimental conditions for intervals ranging from 1 to 96 hours.

Results

Oxygen consumption of seedlings germinated aerobically

The seeds used throughout were Sacramento barley, and Carreon Upland rice from the Philippines. Seeds were germinated aerobically between 2 screens, of which the lower was covered by cheese cloth, and the upper by mosquito netting. Moisture was maintained by wick action of the cloth dipping into a pan of water. The details of the procedure have been described previously (**4**).

Germination occurred in a dark room controlled at 25° C. When the seedlings attained a 2-cm. shoot length, they were placed in experimental vessels of the Fenn apparatus (**2**) to measure the oxygen consumption under six oxygen pressures.

Mixtures of oxygen and nitrogen were prepared such as to give partial pressures of 9.5, 5.2, 2.7, 1.0 and 0.2 per cent. oxygen. Atmospheric air provided 21 per cent. oxygen.

The analysis for oxygen was performed by the Winkler method (**13**), and a colorimetric procedure based on oxidation of metallic copper to blue cupric ion (**3**).

For each measurement the seedlings were introduced into the vessel and the oxygen uptake measured at 21 per cent. oxygen until equilibrium was established, usually within one hour. Then one of the gas mixtures was introduced and the oxygen uptake measured for that pressure of oxygen. This provided a method of relating all rates of respiration to those found at 21 per cent. which arbitrarily received a value of 100. All determinations were made in triplicate at 23.5° C.

The curves for rice and barley seedling respiration (oxygen consumption) are shown in figure 1 and appear very similar, giving a steep trend from 0.2 per cent. to 9.5 per cent. oxygen.

Carbon dioxide production of seedlings germinated aerobically

The respiration of seedlings similar to those above was also compared with respect to carbon dioxide production at 21 per cent. and 0.2 per cent.
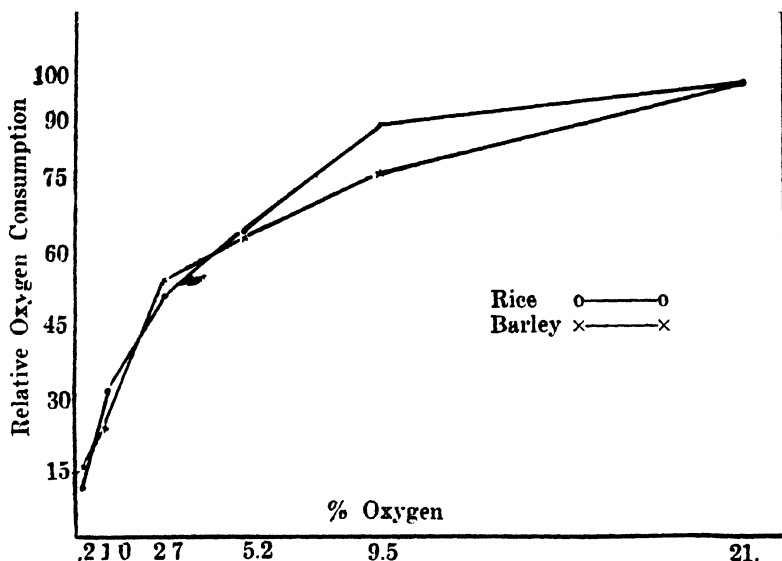
FIG. 1. Relative oxygen consumption under different oxygen tensions of rice and barley seedlings germinated aerobically.

oxygen. This was done by passing the CO_2-free gas under pressure at the rate of 1 liter per hour through a half-liter Erlenmeyer flask containing 25 barley or rice seedlings. A double layer of filter paper at the bottom of the flask retained sufficient moisture to maintain a saturated atmosphere. The time required for a series of determinations was less than 12 hours. The temperature was maintained at 25° C.

The carbon dioxide released was absorbed in KOH towers and titrated to the endpoint of phenolphthalein. The data (table I) show the reduction in respiration of rice and barley seedlings under 0.2 per cent. oxygen. For both plants this amounts to about 50 per cent. of the CO_2 evolved under 21 per cent. oxygen.

GERMINATION AND GROWTH AT DIFFERENT OXYGEN TENSIONS

The preceding experiments measured the oxygen sensitivity of barley and rice seedlings germinated under the usual aerobic atmosphere. A

TABLE I

ANAEROBIC CO_2 PRODUCTION AT 25° C.

TYPE OF RESPIRATION	BARLEY	RICE
Aerobic (21% oxygen)	100	100
Anaerobic (0.2 % oxygen)	50.0	49.5
	54.5	49.0
	52.6	56.5
	51.5	50.0
	52.5	53.8
Average anaerobic	52.2	51.8
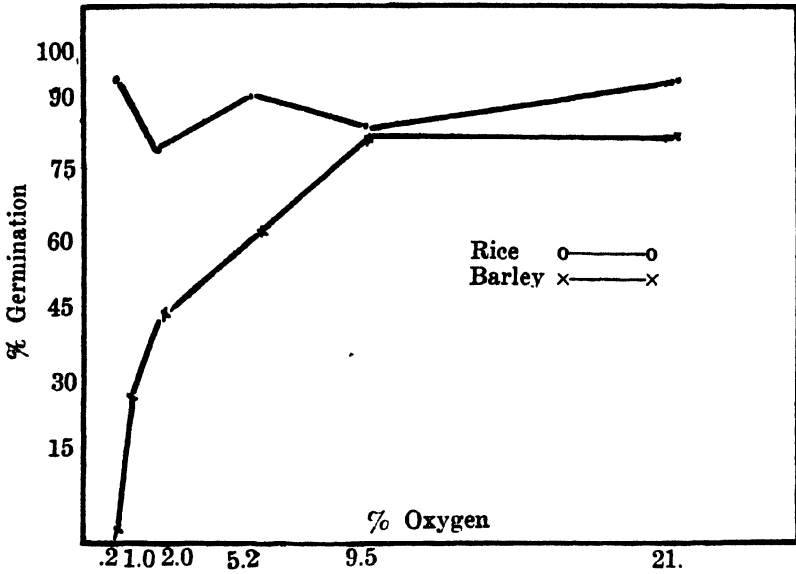
FIG. 2. Germination of rice and barley seeds in various mixtures of oxygen and nitrogen renewed every 24 hours.

further comparison was undertaken to germinate seeds under several partial pressures of oxygen from the earliest stage of germination.

Twenty-five dry seeds were placed on 2 filter papers in each of six 500-ml. Erlenmeyer flasks equipped with inlet and outlet tubes. Each flask was filled with distilled water saturated at the required oxygen pressures.

After 18 hours the water was displaced by a known oxygen-nitrogen mixture under pressure, leaving sufficient water to maintain the filter moist,
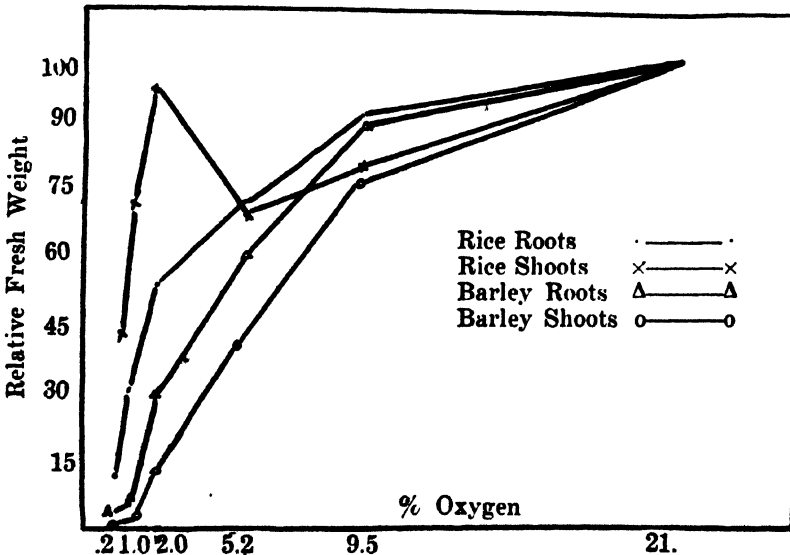
FIG. 3. Relative root and shoot fresh weight in oxygen-nitrogen mixtures renewed every 24 hours.

while avoiding any seed submersion. The atmosphere thus provided was renewed every 24 hours so that a fresh supply of the gas mixture was available once daily. The temperature was kept constant at 25° C.

Barley seedlings were harvested after a period of 5 days, while the rice was grown for 11 days. This difference in time was imposed by the slower rate of development in rice.

The percentage of germination for barley (fig. 2) is not unlike the oxygen consumption curve of barley seedlings germinated aerobically (fig. 1). The germination of rice shows a contrasting reaction to low oxygen pressure, and the percentage of seed germinated is independent of oxygen tension.

The growth values (fig. 3) are based relatively on those at 21 per cent. oxygen which are taken as 100. The root growth curves of rice and barley are very similar although rice does show a slightly higher trend than barley at the lower oxygen values.

The shoot growth of barley is of the same character as the root response, whereas rice shoots show as much growth in 2.0 per cent. oxygen as in 21 per cent.

An unsatisfactory feature of this experiment is to be found in the static atmosphere. Even with daily renewals the accumulation of some carbon dioxide and the inevitable reduction of the initial oxygen content represent a certain uncontrolled variation.

GERMINATION, GROWTH, AND RESPIRATION

In order to meet the objections described above, seeds were germinated in continual streams of gas mixtures with known oxygen content. This automatically provided a means of measuring respiration by absorbing in alkali tubes the carbon dioxide evolved.

The culture chamber again consisted of a half-liter Erlenmeyer flask containing two filter papers and a rubber stopper with 2 glass tubes. On the inlet side provision was made for elimination of any CO_2 in the incoming gas by absorption in strong alkali. The outlet tube led to the standard alkali absorption tower. The rate of aeration equalled 1 liter per hour, which was adequate to maintain the oxygen levels for all mixtures except the 0.2 per cent. (anaerobic) level.

Seeds were placed in the Erlenmeyers and soaked overnight in water aerated with the respective oxygen mixtures. After forcing the liquid from the flasks, the respiration measurements were started immediately and observations made at frequent intervals continuously until the termination of the experiment. Barley was harvested after 5 days and rice after 11 days.

The relative fresh weights are illustrated in figure 4, and show in an accentuated form the data obtained in the preceding experiment. Barley root and shoot growth rapidly declines as the oxygen pressure goes below 9.5 per cent. The rice roots have a slightly lower rate of decrease than barley shoots or roots, but the difference is not enough to be of great significance.
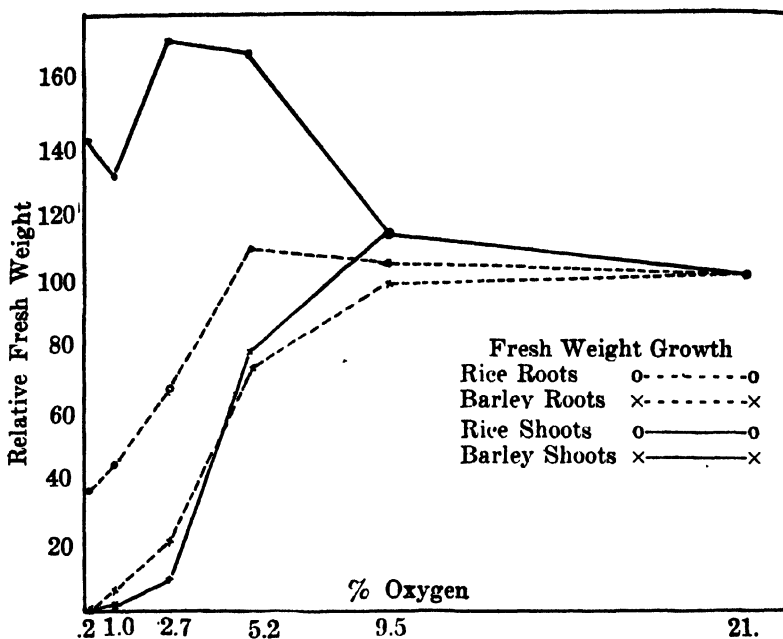
FIG. 4. Relative root and shoot fresh weight in continuous streams of oxygen-nitrogen mixtures.

The rice shoots at all pressures ranging from 0.2 per cent. to 5.2 per cent. oxygen are considerably larger than at the 2 highest pressures. In
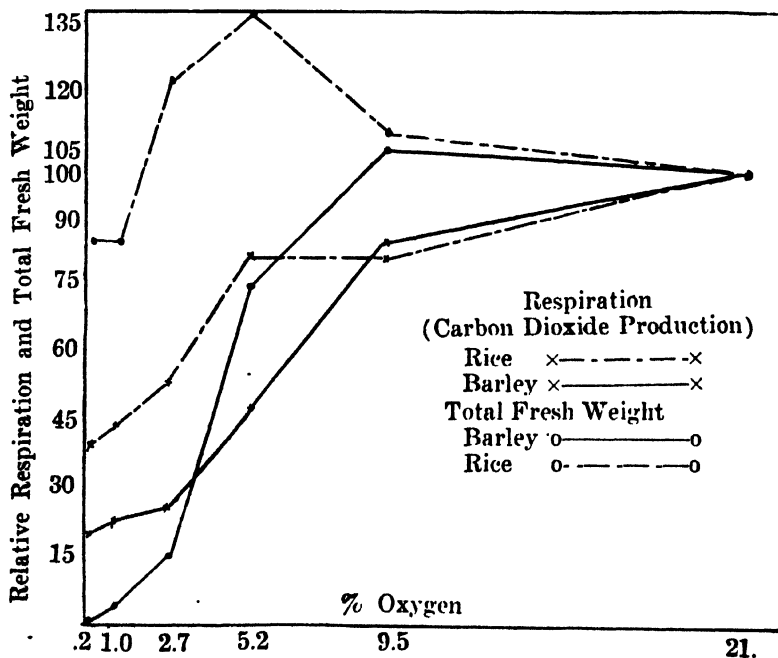
FIG. 5. Total carbon dioxide evolved and total fresh weight in continuous streams of oxygen-nitrogen mixtures.

fact, the highest oxygen tension (21 per cent.) actually yielded the minimum value in shoot growth, although the difference from the next value (9.5 per cent. oxygen) is not very large.

The respiratory quantities express the total carbon dioxide production throughout the germination and growth of the seedlings (fig. 5). The barley curve follows the previous patterns while that of rice is slightly higher than barley at the four lowest oxygen pressures. Some such relation would be anticipated on the basis of the differences found in growth at low oxygen tensions, especially with reference to the rice shoots.

The total growth is also presented to show the relation of total respiration to the combined root and shoot growth. Further calculations to estimate the respiration per unit growth were not made in the absence of any assessment of the respective respiratory power of the shoot and root under low oxygen tensions.

It is obvious that the rice seedlings germinated under low partial pressures of oxygen exhibit a dual character. This expresses itself on the one hand by the usual inhibiting effect on the development of the roots which is comparable to that occurring in barley root and shoot growth. On the other hand is found an apparent stimulation of the shoot system which results in a considerable increase over that undergoing aerobic germination.

This dichotomy can hardly be explained by any such simple device, for example, that has implied an increased availability of some reserve substances which are released to the shoots through the inability of the roots to utilize them. In a similar category is the prospect that a substance is produced by the roots under oxygen deficiency and increases the growth of the shoot at low oxygen pressures. Neither explanation provides a clue as to the origin of the unique behavior of the shoot system in the first place.

Any attempt to elucidate the mechanism as its primary aim must measure the anaerobic response of root and shoot separately without sacrificing too greatly in regard to time, the inter-relationships involved. In addition to the oxygen and carbon dioxide quantities exchanged, the effects of some anaerobic intermediates and end-products likewise require exploration in the hope of identifying the metabolic chain responsible for the behavior of the rice shoot at low oxygen tension. Possible toxicity effects on the shoots at the higher pressures also merit some attention.

Summary

Barley and rice seeds germinated aerobically were tested for respiratory behavior over short time intervals under partial pressures of oxygen ranging from 0.2 per cent. to 21 per cent. For both plants there is an equally rapid decline in oxygen consumption as the pressure goes below 9.5 per cent. Measured by carbon dioxide production, the respiratory activity of barley and rice at 0.2 per cent. oxygen is approximately half that at 21 per cent.

Seeds were germinated under various oxygen pressures and continuously aerated with oxygen-nitrogen mixtures up to the time of harvesting. The carbon dioxide released throughout this time was measured periodically.

Barley root and shoot growth is considerably diminished below 9.5 per cent. oxygen. Rice roots undergo a smaller reduction in growth rate than barley roots. Rice shoot growth is at a minimum at 21 per cent. and increases with declining oxygen pressure, reaching a maximum at approximately 3 per cent. Below this pressure the growth of rice shoots is still significantly higher than at 21 per cent.

The rate of evolution of carbon dioxide by rice decreases with lowered oxygen pressure and that by barley declines more rapidly.

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LITERATURE CITED

1. ERYGIN, P. S. Change in activity of enzymes, soluble carbohydrates, and intensity of respiration of rice seeds. Plant Physiol. **11**: 821–832. 1936.

2. FENN, W. O. A new method for the simultaneous determination of minute amounts of carbon dioxide and oxygen. Amer. Jour. Physiol. **84**: 110–118. 1928.

3. HAEHNEL, W., and MUGDAN, M. Über die Sauerstoffbestimmung mit Kupferammoniak. Zeitschr. angew. Chem. **33**: 35. 1920.

4. HOAGLAND, D. R., and BROYER, T. C. General nature of the process of salt accumulation by roots with description of experimental methods. Plant Physiol. **11**: 471–507. 1936.

5. JONES, J. W. Effect of depth of submergence on the control of barnyard grass and the yield of rice grown in pots. Jour. Amer. Soc. Agron. **25**: 578–583. 1933.

6. MACK, W. B. The relation of temperature and the partial pressure of oxygen to respiration and growth in germinating wheat. Plant Physiol. **5**: 1–68. 1930.

7. MORINAGA, T. Catalase activity and the aerobic and anaerobic germination of rice. Bot. Gaz. **79**: 73–84. 1925.

8. ——————. Germination of seeds under water. Amer. Jour. Bot. **13**: 126–140. 1926.

9. ——————. The favorable effect of reduced oxygen supply upon the germination of certain seeds. Amer. Jour. Bot. **13**: 159–166. 1926.

10. NAGAI, I. Some studies on the germination of the seeds of *Oryza sativa*. Jour. Coll. Agr., Imp. Univ. Tokyo **3**: 109–158. 1916.

11. TAKAHASHI, T. Is germination possible in absence of air? Bull. Coll. Agr., Imp. Univ. Tokyo **6**: 439–442. 1905.

12. TAYLOR, D. L. Effects of oxygen on respiration, fermentation, and growth in wheat and rice. Science n.s. **95**: 129–130. 1942.

13. TREADWELL, F. P. Analytical chemistry. Vol. 2. p. 760. 1912.

14. YOKOI, T. On the development of the plumule and radicle of rice seed with various quantities of water in the germinating medium. Bull. Coll. Agr., Imp. Univ. Tokyo **3**: 482–487. 1898.

FIRMNESS OF STRAWBERRIES AS MEASURED BY A PENETROMETER

LELAND BURKHART

(WITH TWO FIGURES)

Introduction

Strawberries are subjected to compression, bruising, and shearing effects during harvesting, packing, and shipping. The resistance of the fruit to mechanical injury is related to firmness; hence there is needed a suitable method for measuring firmness in order to evaluate varietal differences, stage of maturity, or degree of ripeness, effects of environmental conditions during fruit development on firmness, and effects of different shipping and storage conditions on changes in firmness.

The improved types of pressure testers (**6, 10**) designed for pomaceous fruits are not sufficiently sensitive for evaluating differences in firmness of strawberries. No satisfactory pressure tester has been reported for use on strawberry fruit (**6**). Resistance of strawberries to wounding was measured by HAWKINS and SANDO (**8**) as the pressure required to puncture the epidermis with a needle 636 microns in diameter. CULPEPPER *et al.* (**3**) measured the resistance of the strawberry to penetration by a small needle (0.032 inch in diameter). The depth of penetration was not indicated, however; the sudden penetration of the needle was used as the end point. This needle tester gave an index of maturity but did not measure varietal differences in firmness or resistance to crushing in shipping or handling. This device did not give a measure of the resistance of the first to deformation or flattening as does the pressure or squeeze tester developed by HALLER *et al.* (**7**). This tester which measured the resistance to flattening of the fruit is a modification of the type described by VERNER (**14**). A 2,000-gram scale was used and the plunger disc moved through a distance of ⅛ inch. Full ripe berries of approximately the same stage of maturity were used. A direct correlation was found to exist between the dry weight and firmness of the different varieties.

In order to determine the effect of moisture on the softness of berries, KIMBROUGH (**9**) used a sweet-corn tester, with a plunger ¼-inch in diameter which registered in grams the pressure needed to penetrate the berry. Berries from watered plants were larger and softer than from the unwatered plants.

SHOEMAKER and GREVE (**13**) adapted an apple pressure tester to strawberries. A plunger ¼-inch in diameter was used. Readings were taken when the plunger had been forced ⅜-inch into the berry at a point one third the distance from the calyx to the apical end. The end point of penetration was indicated by electrical contacts. Firmness increased through the season, June 11th to 16th, and this was attributed to the smaller size of the berry.

CLARK (4) used a pressure tester with a plunger $\frac{5}{16}$-inch in diameter for ripe berries. Force was applied by hand until the plunger penetrated $\frac{1}{8}$-inch into the berry. DARROW (5) used this tester and found that fertilizers had no effect on firmness of strawberries.

One of the chief disadvantages of these previously reported pressure testers is that the applied force is not mechanically controlled.

Strawberry penetrometer

The improved type of penetrometer as shown in figure 1 and described herein is designed to include a controlled application of the pressure necessary for fruit penetration by the plunger. The pressure was applied by the delivery of 30-mesh zinc metal from a funnel burette, at a uniform rate of 10 grams per second, to a container resting on the platform of a spring
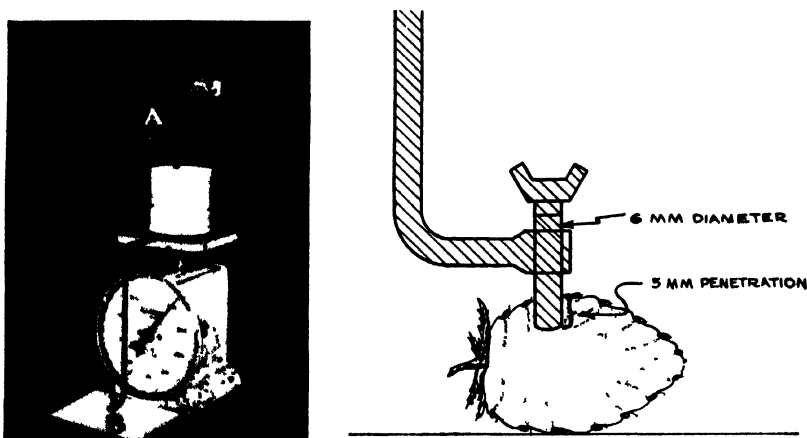
FIG. 1. Left: Photograph of the penetrometer showing controlled uniform rate of application of force at A which is transmitted to the strawberry fruit placed under the plunger at B. The force is applied as 30 mesh zinc metal delivered from the funnel burette at A. Right: Diagram showing fruit penetration by the penetrometer plunger.

balance of 1000-grams capacity. The applied force is transmitted from the platform to the fruit plunger 6 mm. in diameter by means of a steel rod fastened to the platform. On the basis of the structure of the fruit and observations that tissue breakdown occurs initially in the cortical region of the fruit, a penetration of 5 mm. proved satisfactory in the vulnerable zone of greatest diameter which is one-third the distance from the calyx to the apical end. The distance of 5 mm. traversed by the plunger into the fruit is conveniently indicated by the deflection of the pointer on the dial from the zero reading of 100 to the dial reading 250. The dial reading of 250 is the endpoint of the determination obtained by immediately stopping the flow of zinc by releasing the pinchcock. The firmness of the fruit is indicated by the weight of zinc required to overcome the resistance of the fruit to plunger penetration which is obtained after subtracting the resistance of the balance to the passage of the plunger through air.

Results

Variations in the chemical characteristic ascorbic acid (2) in sun-ripened and shade-ripened strawberries suggested the possibility that wide variation might occur in firmness as a physical characteristic of fruit grown under these different light conditions.   There is shown in table I the effect of direct illumination and shading of foliage on large, medium, and small Klondike strawberries.   The fruit was picked for uniformity as to visual red color. Strawberries ripening in direct sunshine tend to be more firm than the shade-ripened fruit, and this tendency is consistent in the large, medium, and small fruits.   Under these conditions of sunshine and shade, the larger

TABLE I

STRAWBERRY PRESSURE TESTS.   VARIATION IN FIRMNESS WITHIN AND BETWEEN
SUN-RIPENED AND SHADE-RIPENED KLONDIKE STRAWBERRIES PICKED
FROM AN EXPERIMENTAL FERTILITY PLOT*

	BERRY 1	BERRY 2	BERRY 3	BERRY 4	BERRY 5	BERRY 6	MEAN
	F†	F	F	F	F	F	F
	gm.	gm.	gm.	gm.	gm.	gm.	gm.
Sun-ripened							
Large	90– 96	128–143	135–167	143–159	148–155	158–181	142.9
Medium	80–128	100–128	134–164	140–150	148–158	148–160	137.0
Small	70– 72	68–100	148–159	148–170	150–154	166–178	131.8
Shade-ripened							
Large	64– 91	86–110	97–107	117–125	131–160	161–174	118.5
Medium	76– 86	72– 98	86– 96	94–102	114–124	180–188	109.6
Small	68– 70	73– 80	88– 94	96–110	102–118	110–130	95.3

* Picked May 9; analyzed May 10.
† Firmness of each berry is indicated by two determinations made at a right angle to each other in the same transverse plane.

berries appear more firm than the smaller ones, which is not in agreement with the findings of DARROW (5).   The largest variations in firmness occurred, however, between individual fruits under both light conditions and within a fruit as shown in table I.   A more detailed report of the factors associated with these wide variations between fruits and within a fruit will appear in a later publication.   These variations in firmness within typical samples of different varieties are shown in table II and the need of further study of the internal variations in samples is suggested.   The mean values of firmness indicate wide varietal differences.   It is possible that firmness values greater than 200 grams may indicate better shipping and keeping qualities than values below 200 grams.   The possibilities of the penetrometer for measuring quantitative differences in firmness of new seedling strawberries developed in breeding programs is indicated.   Seedling 3911–13 has a mean firmness value 200 per cent. greater than seedling 3805–06.

Firmness of strawberries as related to stages of maturity or degree of ripeness on the basis of visual red color intensification is shown in figure 2. In the early stages of red coloration, firmness of the fruit decreased sharply.

TABLE II

VARIATION IN FIRMNESS WITHIN AND BETWEEN RIPE STRAWBERRIES OF MEDIUM SIZE IN
TYPICAL SAMPLES OF DIFFERENT VARIETIES*

VARIETY	BERRY 1	BERRY 2	BERRY 3	BERRY 4	BERRY 5	BERRY 6	MEAN
	F†	F	F	F	F	F	F
	gm.	gm.	gm.	gm.	gm.	gm.	gm.
Seedling 3803–06	58– 70	68–114	84– 88	88–108	68–134	132–144	96.5
Klondike	100–106	102–108	104–118	113–121	124–136	130–132	116.1
Missionary	110–133	122–129	128–150	152–159	158–165	178–180	147.0
Blakemore	232–248	232–252	212–270	232–276	228–302	252–278	251.1
Seedling 3911–13	234–276	250–264	266–274	298–318	302–338	344–350	292.8

* Picked April 29, 1942; analyzed April 30, 1942.
† Firmness of each berry is indicated as two determinations made at a right angle
to each other in the same transverse plane.

After reaching the pink stage the Klondike variety decreased more rapidly
than the Fairmore. The firmness of the Fairmore variety is more stable
during ripening than the Klondike variety. A marked varietal difference
in firmness of plums as related to maturity on the basis of color change has
been reported (7). In connection with the evaluation of stages of maturity
on degrees of ripeness, there is also needed a quantitative measurement of
the red color or anthocyanin concentration. As the strawberries reach an
over-ripe condition, the internal tissue of the fruit breaks down, resulting in
rupture of the skin and it is at that stage when the ascorbic acid content of
the fruit is rapidly destroyed (10).
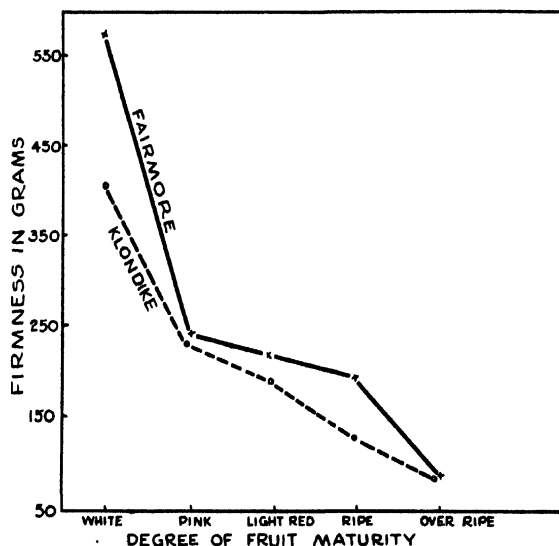
FIG. 2. Firmness of Fairmore and Klondike varieties of strawberry fruit at different
stages of fruit maturity as indicated by red color intensity.

The use of the penetrometer in evaluating effects of storage conditions on keeping and shipping quality of strawberries is suggested by the firmness values shown in table III. The low storage temperature increased firmness, and in this connection HAWKINS and SANDO (8) found that low temperatures increased the resistance of the strawberry epidermis to puncturing. The need of a refined sampling technique to increase the reliability of the firmness determinations for evaluating treatment differentials is indicated by the variation in the mean values of the respective samples which received the same fertilizer treatment.

TABLE III

EFFECT OF STORAGE TEMPERATURE ON FIRMNESS OF RIPE BLAKEMORE STRAWBERRIES SAMPLED FROM AN EXPERIMENTAL FIELD*

	APRIL 25	APRIL 27 AFTER 48 HR. AT 8° C.	APRIL 27 AFTER 48 HR. AT 27° C.
	gm.	gm.	gm.
Sample A	188.5	234.6	132.1
Sample B	195.6	227.4	115.8
Sample C	204.5	230.5	125.8
Sample D	196.5	217.1	128.1
Mean	196.0	227.5	123.0

* Picked April 24. Each value represents the mean of twelve determinations.

Summary

1. The improved penetrometer for measuring the firmness of strawberries is designed to include a controlled uniform rate of application of the force necessary for fruit penetration by the plunger. A penetration depth of 5 mm. with a plunger 6 mm. in diameter proved satisfactory. The firmness of the fruit is indicated by the force required to overcome the resistance of the fruit to penetration by the plunger.

2. Strawberries ripened in sunshine tend to be more firm than shade-ripened fruit. Larger berries appeared more firm than the smaller.

3. Varietal differences in firmness are indicated and ranked in the following descending order: Blakemore, Massey, Missionary, and Klondike. Two new seedling varieties differed in firmness to the extent of 200 per cent.

4. Changes in firmness of Klondike and Fairmore strawberries during ripening are indicated.

5. Wide variations in firmness occurred among fruits within samples and within individual fruits. These observations indicate the need of a refined sampling technique to increase the reliability of the firmness determinations for evaluating treatment differentials. The use of the penetrometer in evaluating changes in firmness under different storage conditions is indicated.

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LITERATURE CITED

1. ALLEN, F. W., MAGNESS, J. R., and HALLER, M. H. The relation of maturity of California plums to shipping and dessert quality. California Agr. Exp. Sta. Bull. 428. 1927.

2. BURKHART, L., and LINEBERRY, R. A. Determination of vitamin C and its sampling variation in strawberries. Food Res. 7: 332–337. 1942.

3. CULPEPPER, C. W., CALDWELL, JOSEPH S., and MOON, HUBERT H. A physiological study of development and ripening in the strawberry. Jour. Agr. Res. 50: 645–696. 1935.

4. CLARK, J. H. Pressure tests. of strawberries. New Jersey Agr. Exp. Sta. Ann. Rept. 1928: 227. 1928.

5. DARROW, GEO. M. Effect of fertilizers on firmness and flavor of strawberries in North Carolina. Proc. Amer. Soc. Hort. Sci. 28: 231–235. 1931.

6. HALLER, M. H. Fruit pressure testers and their practical applications. U. S. Dept. Agr. Circ. 627. 1941.

7. —————, HARDING, P. L., and ROSE, DEAN H. The interrelation of firmness, dry weight, and respiration in strawberries. Proc. Amer. Soc. Hort. Sci. 29: 330–334. 1933.

8. HAWKINS, LON A., and SANDO, CHARLES E. Effect of temperature on the resistance to wounding of certain small fruits and cherries. U. S. Dept. Agr. Bull. 830. 1920.

9. KIMBROUGH, W. D. The quality of strawberries as influenced by rainfall, soil moisture and fertilizer treatments. Proc. Amer. Soc. Hort. Sci. 27: 184–186. 1931.

10. LINEBERRY, R. A., and BURKHART, LELAND. Stability of vitamin C in small fruits. Fruit Products Jour. 22: 164–165. 1943.

11. MAGNESS, J. R., and TAYLOR, GEORGE F. An improved type of pressure tester for the determination of fruit maturity. U. S. Dept. Agr. Circ. 350. 1925.

12. ROSE, D. H., HALLER, M. H., and HARDING, P. L. Relation of temperature of fruit to firmness in strawberries. Proc. Amer. Soc. Hort. Sci. 32: 429–430. 1935.

13. SHOEMAKER, J. S., and GREVE, E. W. Relation of nitrogen fertilizers to the firmness and composition of strawberries. Ohio Agr. Exp. Sta. Bull. 466. 1930.

14. VERNER, LEIF. Experiments with a new type of pressure tester on certain stone fruits. Proc. Amer. Soc. Hort. Sci. 27: 57–62. 1931.

SPECTROGRAPHIC ANALYSIS OF PLANT ASH FOR SEVERAL ELEMENTS SIMULTANEOUSLY[1]

R. C. NELSON, P. C. HAMM, AND Y. S. TSIANG

(WITH TWO FIGURES)

Introduction

For studies of the relative mineral nutrient absorptive capacities of different plant species or strains under varying environmental conditions, a method of determining all the elements of interest in a single series of operations is a considerable convenience. The nature of quantitative spectroscopy suits it well to the solution of such problems. Important difficulties arise, however, in the standardization of the variations in burning the sample and making the photographic record; and in the uncertainties due to the interaction of the elements of the sample. The first of these problems is ordinarily met by the use of an internal standard, and the second by comparison of the unknown with preparations of similar composition made artificially. In the matter at hand, both of these devices are unduly laborious.

Theoretical considerations

Internal standardization usually involves the addition of a known quantity of an element not present in the sample, and the use of lines so obtained as density standards for the estimation of unknown quantities of other elements. This succeeds only when the line of the standard and the line of the unknown which is compared with it are activated under the same conditions and are affected similarly by variations in the arc and in composition of sample; and when the two elements in question volatilize simultaneously during the burning of the sample. The first condition may be met by a pair of lines which are produced by corresponding shifts in their respective atoms. The second may be tested by the use of moving plate spectrograms, which reveal the relative times at which the elements in the sample pass into the arc.

These conditions may usually be met, but often only with difficulty, and the use of a separate standard for each of six elements in an analysis would be troublesome. In a spectrograph of medium dispersion such a procedure would lead to a crowded spectrum, owing to the lines of the extraneous elements introduced.

At a sacrifice of some accuracy, and with the additional loss either of economy or sensitivity, this difficulty may be overcome by dispensing with the internal standard, and altering the other conditions of the analysis so as to reduce the variability or compensate for it. BRUNSTETTER, MYERS, WILKINS, and HEIN (1) accomplished this by adjusting the conditions of

the burning of the sample so that they were reproducible, with a concomitant loss of sensitivity.

The instrument used in the present experiments, that described by NEL-SON (3) is not sufficiently sensitive to permit this solution, but its economy of operation as regards photographic materials is such that it is possible to increase the number of exposures made of each sample without making the cost of a determination unduly large. By making four, or even eight exposures for the highest accuracy, it is possible to average out the variations caused by irregularities in the arc, without requiring a great expenditure of either time or money.

The interactions of elements in the arc and the effects of concentration of one upon the density of lines of another are not yet well understood. In
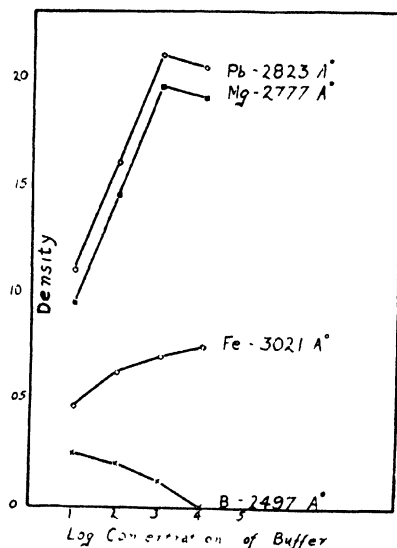
FIG. 1.   Effect of sodium acetate on line density.

the analysis of plant ash the chief difficulty arises from differences in the quantity of sodium and potassium salts present. Figure 1 shows the effect of the addition of increasing amounts of sodium acetate upon the density of certain lines. Increasing the amount of sodium in the sample, under the conditions of these experiments, enhances the density of certain lead and magnesium lines, has a small effect upon a line of iron, and depresses a boron line. Other elements may also cause such effects or be similarly affected. It is thus apparent that the composition of the artificial standard from which the calibration curve is obtained must closely approximate that of the unknown sample. To make such a standard by trial and error would be very laborious. A more convenient method, using the unknown itself to obtain the calibration curve will be described.

Let x be the concentration of an element in the unknown, expressed in micrograms per milliliter. By taking a series of different volumes of the unknown solution, containing V_1x, V_2x, etc., micrograms of the element, it

is possible to obtain a calibration curve in terms of x. If, now, a known quantity of the element is added to V_1x, the density of the line will be increased to an extent corresponding to some determinable point on the calibration curve, V_nx, or $V_1x + y$ micrograms = V_nx, giving an equation which may be solved for x, after which numerical values may be attached to the proportional points of the calibration curve. By this means, the procedure is standardized, using a preparation which is almost identical in composition with the unknown. When a large number of samples of similar composition is being analyzed, a composite of several of them may be used as the unknown in this method. Known amounts of all the elements which are present only in small quantities may be added at once, although obviously
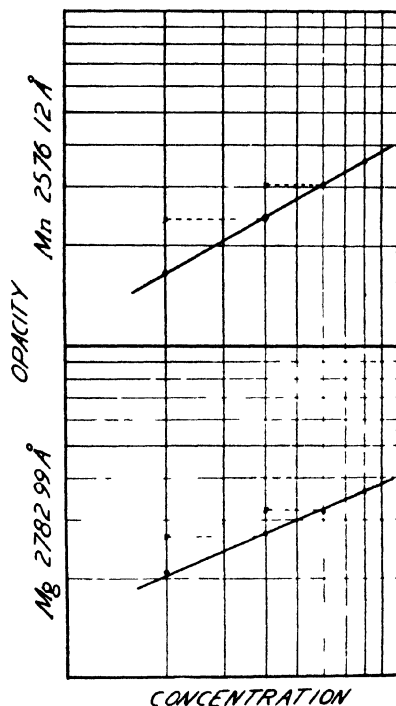
FIG. 2. Standardization curves for magnesium and manganese.

it is preferable not to add potassium to the unknown when standardizing it for the other elements. The value of V_n may be obtained graphically or by solution of an equation fitted to the line density-volume values.

Figure 2 illustrates this method. A curve was made by plotting line density against logarithm of volume of solution taken, using 0.02, 0.04, 0.06, and 0.08 ml. of the unknown and the following equation fitted by the method of least squares.

$$2 + \log V = 0.005 + 1.61 \times \text{density}$$

Then, to 0.02 and to 0.04 ml. of the unknown were added 0.4 micrograms of manganese each. This addition resulted in line densities of 0.366 and 0.480, respectively, corresponding to volumes of 0.0392 ml. and 0.0598 ml. These

data correspond to concentrations of manganese in the sample of 20.8 and 20.2 micrograms per ml., which is good agreement. It is thus possible to perform a spectrographic analysis of an unknown for several elements in a series of simple operations without the necessity of preparing an artificial standard.

Experimentation

The determinations herein described were made on a replica grating spectrograph of medium dispersion, using a DC arc of 40 volts drop across the arc and 25 amperes. The record was made upon Verichrome roll film which was developed 105 seconds in 1:1 D–72 at 18° C., stopped in dilute acetic acid, and fixed with F–5. Although it would have been possible to use a sector disk at the stigmatic focus, it was found that gamma could be controlled well enough by care in development, and no attempt was made to measure the gamma of each film.

One-gram samples of dry plant material were ashed, and the ash extracted overnight at room temperature with 5 ml. of 3 N hydrochloric acid. 0.04 ml. of this solution was used for each exposure. Electrodes were purified according to the method of ZEITLOW, HAMM, and NELSON (4), after a cup 2 mm. deep and 4.5 mm. diameter had been drilled at one end of each electrode. This cup was first treated with a drop of a five per cent. solution of paraffin in toluene, after which the sample to be analyzed was pipetted into it and dried under a heat lamp. Electrodes were held three-sixteenths of an inch apart during burning, and arcing was continued for four seconds after the sample was all vaporized, as judged by the sound of the arc. Line densities were read using a densitometer designed for use with roll film and constructed in this laboratory.[2] Four exposures were made of each sample. Occasional exposures which were obviously weak across the whole range of wavelengths, indicating a gross derangement of the arc during burning, were eliminated by inspection, and not considered in calculation of results.

Discussion

The methods here described were developed specifically for application to the analysis of 36 clonal lines of common bromegrass (*Bromus inermis* Leyss.) which were being studied by Y. S. TSIANG. Results of this work will be published later, but some purely analytical aspects of this study will be mentioned here. The following table shows the mean squares of errors due to the analytical procedure compared with the mean squares of total experimental error, computed according to the method of analysis of variance (2).

ELEMENT	WAVELENGTH OF LINE	TOTAL ERROR	SPECTROGRAPHIC ERROR
Fe	3020 A.	220.79	2.46
Cu	3247	13.52	8.23
Ca	3158	1,654,441.0	111.0
Mn	2576	155.67	0.88
K	3446	1,151,410.0	215.0
Mg	2782	39,111.0	257.0

[2] NELSON, R. C., and HAMM, P. C. Unpublished.

It will be seen that the error in determinatioñ of copper is relatively large, owing probably to the difficulty of removing copper from the carbons in the purification process. BRUNSTETTER *et al.* also found this to be true. Boron and aluminum might also have been determined, but were not of interest; knowledge of the phosphorus content would have been valuable, but phosphorus was not present in large enough quantity to permit reliable determination. There were seventy-two samples of each of which four exposures were made, using nine rolls of V–620 film having thirty-two exposures per roll.

These procedures have been used in other similar problems and have proved quite satisfactory. It must always be remembered, however, that each type of spectrograph and each type of sample require especially adapted procedures to secure the best results.

Summary

A method is described for determining a number of elements simultaneously in ash samples using an increased number of exposures, simple density measurements without internal standardization, and a procedure for establishing a calibration curve without the use of an artificial standard. This technique was adapted to the analysis of samples of brome grass for iron, manganese, copper, calcium, potassium, and magnesium.

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LITERATURE CITED

1. BRUNSTETTER, B. C., MYERS, A. T., WILKINS, H. L., and HEIN, M. A. Simultaneous quantitative determination of seven elements in grasses and legumes spectrographically. Proc. Sixth Summer Conf. Spectroscopy and Its Appl. New York. 1939.
2. FISHER, R. A. Statistical methods for research workers. 8th ed. Oliver and Boyd. Edinburgh, 1938.
3. NELSON, R. C. A grating spectrograph for mineral nutrition studies. Plant Physiol. **17**: 492–496. 1942.
4. ZEITLOW, R., HAMM, P. C., and NELSON, R. C. The purification of spectrographic carbons. Science n. s. **94**: 438. 1941.

DECOMPOSITION OF THE LEAVES OF SOME FOREST TREES UNDER FIELD CONDITIONS[1]

FELIX G. GUSTAFSON

(WITH ONE FIGURE)

In the main the few studies that have been made on the decomposition of leaves have either been made under highly artificial conditions (5) or else over a short period of time (1, 3, 4). Therefore an experiment intended to supply natural conditions as nearly as possible and lasting a number of years was set up in the fall of 1934.

Leaves of sugar maple, hickory (several species), white oak and black oak (several species) were collected from the ground during the period of leaf fall; needles from the red pine were collected from the tree, using needles ready to fall. Weighed quantities of air dry leaves were placed in wire baskets $24 \times 24 \times 6$ inches made from galvanized wire. Half-inch mesh was used for all leaves except pines for which quarter-inch mesh was used to keep the needles from falling out. All baskets were supplied with a cover made from the same material as the rest of the basket. The baskets were placed in the field on November 17. The pine needles were placed under the trees from which they had been collected. The others were placed in a mixed hardwood stand. Before the baskets with the leaves were put in place all fresh leaves were removed from the ground so that they rested on partly decayed leaves. The baskets were placed on level ground to insure that all parts came in intimate contact with the soil. Besides the single species or mixture of species as indicated above, the oak leaves and pine needles were mixed in the order of 72 per cent. of the former to 28 per cent. of the latter. This was done to simulate mixed stands of hardwood and conifers, and to discover whether there is any foundation for the general belief among foresters that there is more rapid decomposition when pine and hardwood leaves occur together than when alone.

The amount of leaves placed in each basket was not the same except in pine, because the leaves were put in weighed baskets first and then weighed. The pine needles were weighed separately and 400 grams put into each basket. The amount of the other leaves varied from about 200 to 300 grams per basket. This quantity of leaves was undoubtedly larger than that which would normally have accumulated on an area of four square feet during one season. The oven dry weight of corresponding samples of leaves were made so that every year comparisons could be made between oven dry leaves.

At the time of setting up the experiments it was thought that maple and hickory leaves would decay very rapidly and therefore only four baskets were made up of these leaves, whereas eight baskets of the others were prepared. The original intention was to remove a basket each spring and fall,

[1] Paper from the Department of Botany of the University of Michigan, no. 822.

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but it was soon discovered that if this were done the supply would be used up long before the leaves had completely decayed; therefore, longer intervals were used in later years.

Due to the difficulty of having the baskets made, about a month elapsed between gathering the leaves and placing them out, and during this period they remained in an air dried condition. As there was no appreciable rain until November, there was perhaps little or no delay in initiation of decay during the first fall.

In the fall of 1935 large meshed wire netting was placed over the leaves in each basket and fresh leaves of the proper type put on top to simulate the fall of new leaves. No more leaves were added after this.

Considerable difficulty was encountered in excluding new pine needles as they could easily fall through the top of the basket. The presence of the

TABLE I

DECOMPOSITION OF LEAVES IN PERCENTAGE OF THE ORIGINAL MATERIAL

PLANT	MAY 18, 1935	OCT. 12, 1935	MAY 24, 1936	OCT. 23, 1936	JUNE 2, 1937	OCT. 28, 1938	APRIL 26, 1942
	%	%	%	%	%	%	%
Hickory	15.0	37.4	53.1*	37.0			
Sugar maple	17.2	30.0	26.9	31.9			
White oak	9.0	16.6	37.4*	28.4	20.0	24.5	63.3
Black oak	10.5	19.9	23.5	24.9	27.4	24.8	46.1
Oak and pine	13.3†	24.8	23.1	26.4	28.0	44.9	59.6
Red pine	5.7	19.7	18.4	21.9	24.3	38.3	

* These figures are way out of line but there is no adequate explanation for them.

† In this sample the oak and pine leaves were weighed separately and loss calculated separately. The pine lost 8.25 per cent. and the oak 16.3 per cent., which are greater losses than when they were in separate baskets.

top layer of needles helped to some extent but undoubtedly needles were included at times which had not been part of the original material. Some difficulty was also encountered in later years with other species as after the top layer of leaves had undergone considerable disintegration it was not possible to remove every bit of these leaves as some would fall through the coarse netting. Another difficulty was the introduction of soil to the bottom layer by worms working up through the soil, and also the growth of grass and other plants through the leaves. All possible care was exercised but undoubtedly the later collections contained material not included at the beginning, and it is believed that none of the original material was lost except through decay so that the loss recorded is somewhat less than it really was. In some instances tunnels of rodents were found under the baskets which would tend to reduce the decay because of lack of contact between the leaves and the soil. This was especially noticeable in the spring of 1942. The samples brought into the laboratory in the fall of 1937 were lost in the process of drying, by getting too hot and catching fire.

The results are presented in table I. The pine and two hardwoods are also presented graphically in figure 1.

It will be noticed that during the first year there was a greater loss than during any succeeding year. This was no doubt due to the decay of the parenchyma tissue. As had been expected, the hickory and maple leaves decayed much more rapidly than those of the oaks; pines, on the other hand, decayed very slowly. No information on the composition of hickory and maple leaves has been found, but WAKSMAN states that oak and pine have a high hemicellulose, cellulose, and lignin content, which would make them very resistant to bacterial action.
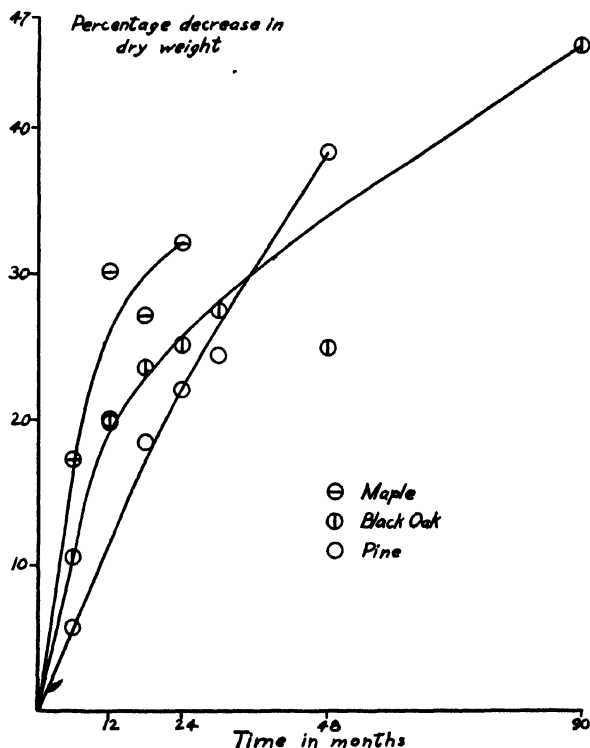
FIG. 1. A graph showing the progressive decomposition of the leaves of red pine, sugar maple, and black oak.

In the 1942 collection the black oak remnant consisted mostly of petioles. The white oak material was composed of recognizable leaf material as well as some petioles. The mixture of pine and oak had the pine needles well disintegrated, and so were the oak leaves.

Figure 1 brings out a difference in the loss of material between the hardwood leaves and the pine needles which is surprising, but easily explained. In the former, the slope of the curve flattens out after the first few collections, whereas the curve for the pine continues at about the same slope. The early rapid disintegration in the hardwood leaves is due to the less resistant parenchyma tissue; but this leaves behind the veins and petioles composed extensively of lignified cells, which decay much more slowly. The

pine on the other hand does not have an extensive parenchyma system distinct from the veins; and the rate of decay is never very rapid, but continues at much the same rate.

The data favor the assumption made by foresters that a mixture of coniferous and hardwood leaves hastens the decay of both. It was not possible, except in the first lot, to determine separately the loss in the two components; but this showed that both the pine and oak leaves lost more weight than when they were in separate baskets. The percentage loss is also greater, year by year, than the oak and pine separately. As an explanation for this might be offered the fact that decaying pine needles produce an acid reaction, which would suppress bacterial activity, but increase fungal activity. The presence of oak leaves with their high calcium content (2) would neutralize the decomposing material, and both bacteria and fungi would be active. The two acting together might decompose more material than fungi or bacteria alone.

Summary

While the experiment is admittedly imperfect, it nevertheless gives us some new information concerning the decay of some hardwood leaves and pine needles over a period of several years. It shows that such leaves as sugar maple and hickory decay much more rapidly than oak, and that oak leaves decay rapidly as long as parenchyma is concerned, but the large veins and petioles last many years. A mixture of pine and hardwood leaves increases the rate of decay of both kinds of leaves.

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LITERATURE CITED

1. FALCONER, J. G., WRIGHT. J. W., and BEALL, H. W. The decomposition of certain types of forest litter under field conditions. Amer. Jour. Bot. **20**: 196–203. 1933.
2. GARSTKA, W. U. The calcium content of Connecticut forest litter. Jour. Forestry **30**: 396–405. 1932.
3. LUNT, H. A. Effect of weathering upon dry matter and composition of hardwood leaves. Jour. Forestry **33**: 607–609. 1935.
4. MELIN, E. Biological decomposition of some types of litter from North American forests. Ecology **11**: 72–101. 1930.
5. WAKSMAN, S. A., TENNEY, F. C., and STEVENS, K. R. The role of microorganisms in the transformation of organic matter in forest soil. Ecology **9**: 126–144. 1928.

INTERNAL PRECIPITATION OF PHOSPHORUS IN RELATION TO ALUMINUM TOXICITY[1]

Kenneth E. Wright

Various investigators (2, 4, 5, 6, 7, 8, 10, 15) have demonstrated that the toxic effects produced in certain plants grown on acid soil are caused primarily by the presence of aluminum in the soil. Poor growth of the plants has been attributed to a phosphorus deficiency caused by the precipitation of phosphorus in the soil by aluminum (3, 5, 6, 8, 10, 11, 12, 14). On the other hand, some research (4, 13, 17) has indicated that aluminum may precipitate phosphorus within the plant, and thus make it unavailable for metabolic processes.

As recorded in an earlier publication (20), the writer grew barley in drip culture solutions wherein the root system was divided, each half being placed in separate containers, and receiving different culture solutions. By this method a plant could have access both to aluminum and to phosphorus, and precipitation in the culture solution could be avoided. Analyses for phosphorus and for aluminum in the various plant fractions revealed that the internal precipitation of phosphorus by aluminum evidently plays an important rôle in the poor development of plants grown in contact with aluminum. The findings reported in this paper further support the thesis that aluminum toxicity may in part be caused by the precipitation of phosphorus internally, thus inactivating phosphorus.

Procedure

Barley seeds were germinated on a disc in distilled water. One week later the plants were transferred to a modified HARTWELL and PEMBER (8) culture solution. The modification consisted in increasing the phosphorus to 12 p.p.m., and in adding 1 p.p.m. each of boron and manganese. Two hundred plants were placed in this solution. Three hundred plants were placed in a similar solution to which had been added 8 p.p.m. of aluminum.

TABLE I

PHOSPHORUS CONTENT OF BARLEY WITH VARIOUS EXTRACTING MEDIA

DETERMINATIONS	NORMAL PLANTS			ALUMINUM-TOXIC PLANTS		
	TOPS	ROOTS	WHOLE	TOPS	ROOTS	WHOLE
Dry weight, grams	83.34	16.66	100.00	18.50	8.35	26.85
Total phosphorus, %	0.86	1.07	0.89	0.76	2.30	1.24
Water-soluble phosphorus, %	0.82	0.92	0.84	0.70	0.39	0.60
pH 3 Sulphuric-acid-soluble phosphorus, %	0.87	1.07	0.90	0.75	0.53	0.68
pH 1 Sulphuric-acid-(1%) soluble phosphorus, %	0.84	1.08	0.88	0.80	2.02	1.18

Five weeks later the plants were harvested, dried, and then ground in preparation for analysis.

Total phosphorus, and one per cent. sulphuric-acid-soluble phosphorus were determined by the method described by BERTRAMSON (1). To determine water-soluble phosphorus, and pH 3 sulphuric-acid-soluble phosphorus, a 0.5-gram sample of plant material in 50 ml. of the extracting medium was placed on a mechanical shaker for thirty minutes, filtered, and the amount of phosphorus in the filtrate ascertained by the method of TRUOG and MEYER (19), using a photoelectric colorimeter. The results are recorded in table I.

Discussion

The toxicity of aluminum is demonstrated by the low yield of the plants grown in the culture solution containing aluminum, a reduction amounting to 73.15 per cent.

The percentage of total phosphorus in the aluminum-toxic plants is greater than that of the normal plants. An accumulation of phosphorus in these poorly developed plants indicates that some of the phosphorus is not being used in the necessary metabolic processes. By comparison with the amount of phosphorus in the normal plants there was sufficient phosphorus in the aluminum-toxic plants to produce a larger yield than that obtained.

Attention is called to the distribution of the phosphorus in the plants grown in the culture solution containing aluminum. Although there is about 50 per cent. reduction in the yield of the roots as compared with the normal plants, the amount of phosphorus in the former is 2.3 per cent., in contrast with 1.07 per cent. in the normal roots. This would indicate that a good portion of the phosphorus is precipitated in the root system, and hence becomes unavailable for the growth of the plants.

The data reveal that the water-soluble phosphorus in the low-yield aluminum plants is 0.6 per cent. as compared with 1.24 per cent. of total phosphorus. With a total phosphorus content of 2.3 per cent. in the roots of these plants, better growth could be expected. With but 0.39 per cent. of water-soluble phosphorus present, it is evident, however, that most of the phosphorus is in a form which cannot be translocated to the growing regions of the plant in amounts sufficient for vigorous growth. PIERRE and STUART (17) found that the plant sap from Cos lettuce grown with aluminum in the culture solution contained only from one-fifth to one-seventh as much soluble inorganic phosphorus as those not receiving aluminum. This, in conjunction with other data, led them to believe that aluminum precipitates phosphorus within the plant making it unavailable for utilization by the plant.

If phosphorus is precipitated in the plant as aluminum phosphate, a method which would distinguish between organically and inorganically bound phosphorus should show relatively high amounts of inorganically bound phosphorus in the aluminum plants as compared with the normal plants. Such a method has been described by BERTRAMSON (1). Inor-

ganically bound phosphorus is obtained by extracting with one per cent. sulphuric acid solution. Organically bound phosphorus is determined by the difference between the inorganic fraction and total phosphorus. The results recorded in the table are typical of numerous determinations which were obtained by the writer with this method. In most instances the analyses for total phosphorus and for inorganic phosphorus were very similar. Whatever differences were obtained were hardly significant, nor did the results show a consistent trend. Organic phosphorus was a small negative quantity as often as a small positive quantity.

In order to find an extracting medium which might differentiate between the phosphorus combined with aluminum, and phosphorus otherwise bound, a series of sulphuric acid solutions varying by units of 0.5 pH from pH 1 to pH 5 was prepared. A 0.5-gram sample of plant material from the tops of the normal barley plants in 50 ml. of each of the nine solutions of acid served as checks against nine similar solutions to which aluminum phosphate as $AlPO_4$ in one group, and as $Al_2(HPO_4)_3$ in another group had been added to the plant material. The solution which just failed to extract phosphate from either of the salts was that with a hydrogen ion concentration of pH 3. PATTEN (16) stated that aluminum phosphate in hydrochloric acid was precipitated between pH 3 and pH 3.5. MAGISTAD's (10) data show that at pH 5 there was an amount of aluminum equivalent to 2 p.p.m. of aluminum oxide in solution, and that there was a rather rapid increase in solubility as the acidity increased beyond pH 4.5. LINE (9) found that aluminum was precipitated as the phosphate from a solution of its salts between pH 3 and pH 4. In extracting phosphorus from soil TRUOG (18) used a sulphuric acid solution buffered with ammonium sulphate at pH 3. This solution extracted "readily available" phosphorus, and did not extract "difficultly available" phosphorus. The latter was considered to exist largely as basic ferric phosphate, and possibly also as aluminum and titanium phosphates. These experiments indicate that an extraction solution of sulphuric acid of pH 3 could be used to determine whether or not there was a difference in the aluminum phosphate content of the barley plants grown in the culture solutions used.

As recorded in table I the amounts of phosphorus extracted from the normal plants by the pH 3 acid are practically identical with the total phosphorus content. In the poorly developed plants grown in the culture solution containing aluminum, however, the amount of phosphorus in the whole plant extracted by the acid is 0.68 per cent., whereas the total phosphorus is 1.24 per cent. The comparison is still more striking when the amount of acid-extracted phosphorus of the roots, 0.53 per cent., is compared with the total phosphorus content of the roots, 2.3 per cent. From this evidence, coupled with the fact that all of the phosphorus was soluble in the sulphuric acid of pH 1 which dissolves aluminum phosphate, it is assumed that the phosphorus which the pH 3 acid failed to extract is combined with aluminum. The inactivation of phosphorus by aluminum principally in the roots

thus creates an actual deficiency of phosphorus in the various meristematic regions of the plant with resultant poor growth.

Summary

Analyses of barley plants grown in culture solutions with and without aluminum indicate that there is a greater percentage of phosphorus in the aluminum-toxic plants than in the normal plants. This accumulation of phosphorus is particularly marked in the roots. A low percentage of water-soluble phosphorus in these plants indicates that the phosphorus is in-activated.

A sulphuric acid solution of pH 3 extracted practically all of the phosphorus from the normal plants, but extracted much smaller quantities from the plants grown in contact with aluminum. The high total phosphorus and low water-soluble phosphorus content of the latter was attributed to the precipitation of phosphorus by aluminum within the plant. This mutual precipitation occurs primarily in the roots, and thus causes a phosphorus deficiency in meristematic regions which is reflected in sharp reductions in yield.

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LITERATURE CITED

1. BERTRAMSON, B. R. Phosphorus analysis of plant material. Plant Physiol. **17**: 447–454. 1942.
2. BLAIR, A. W., and PRINCE, A. L. Studies on the toxic properties of soil. Soil Sci. **15**: 109–129. 1923.
3. ——————, and ——————. The influence of lime in vegetable growing. New Jersey Agr. Exp. Sta. Bull. 498. 1930.
4. BURGESS, P. S., and PEMBER, F. R. "Active" aluminum as a factor detrimental to crop production in many acid soils. Rhode Island Agr. Exp. Sta. Bull. 194. 1923.
5. CONNER, S. D. Liming in its relation to injurious inorganic compounds in the soil. Jour. Amer. Soc. Agron. **13**: 113–124. 1921.
6. ——————, and SEARS, O. H. Aluminum salts and acids at varying hydrogen ion concentrations in relation to plant growth in water cultures. Soil Sci. **13**: 23–43. 1922.
7. GILBERT, B. E., and PEMBER, F. R. Further evidence concerning the toxic action of aluminum in connection with plant growth. Soil Sci. **31**: 267–273. 1930.
8. HARTWELL, B. L., and PEMBER, F. R. The presence of aluminum as a reason for the difference in the effect of so-called acid soil on barley and rye. Soil Sci. **6**: 259–281. 1918.
9. LINE, J. Aluminum and acid soils. Jour. Agr. Sci. **16**: 335–364. 1926.
10. MAGISTAD, O. C. The aluminum content of the soil solution and its relation to soil reaction and plant growth. Soil Sci. **20**: 181–227. 1925.

11. MATHER, WM. The effect of limes containing magnesium and calcium upon the chemical composition of the soil and upon plant behavior. Soil Sci. **13**: 337–354. 1922.

12. MCLEAN, F. T., and GILBERT, B. E. Aluminum toxicity. Plant Physiol. **3**: 293–302. 1928.

13. MCGEORGE, W. T. The influence of aluminum, manganese, and iron salts upon the growth of sugar cane, and their relation to the infertility of acid island soils. Exp. Sta. Hawaiian Sugar Planters' Assoc. Agr. and Chem. Ser. Bull. 49. 1925.

14. MIRASOL, J. J. Aluminum as a factor in soil acidity. Soil Sci. **10**: 153–219. 1920.

15. MIYAKE, K. The toxic action of soluble aluminum salts upon the growth of the rice plant. Jour. Biol. Chem. **25**: 23–28. 1916.

16. PATTEN, A. J. Report on the determination of iron and aluminum, calcium and magnesium in the ash of seeds. Jour. A. O. A. C. **6**: 418–422. 1923.

17. PIERRE, W. H., and STUART, A. D. Soluble aluminum studies: IV. The effects of phosphorus in reducing the detrimental effects of soil acidity on plant growth. Soil Sci. **36**: 211–226. 1933.

18. TRUOG, EMIL. The determination of the readily available phosphorus of soils. Jour. Amer. Soc. Agron. **22**: 874–882. 1930.

19. ——————, and MEYER, A. H. Improvements in the Denigés colorimetric method for phosphorus and arsenic. Ind. & Eng. Chem. Anal. Ed. **1**: 136–139. 1929.

20. WRIGHT, K. E. Effects of phosphorus and lime in reducing aluminum toxicity of acid soils. Plant Physiol. **12**: 173–181. 1937.

BRIEF PAPERS

PYRROLE DERIVATIVES AND IRON CHLOROSIS IN PLANTS

S . A R O N O F F   A N D   G .   M A C K I N N E Y

POLLACCI and ODDO (6) have stated that alpha substituted pyrroles, such as the aldehyde and carboxylic acid may replace iron functionally in chlorophyll-deficient plants. As a corollary, it was believed that the function of iron in green plants was concerned with the formation of pyrrole derivatives possessing a labile alpha substituent. Other authors (2, 3) have not confirmed these experiments. The work was then repeated and the original results re-affirmed (5, 7). Following the negative work of DEMIDENKO (1) the experiments were again repeated by LODOLETTI (4) who extended the theory to include non-homologous water insoluble pyrrolic derivatives such as acetylmethylindole and acetylmethylbenzo-pyrrole. In view of the contradictory reports, we have repeated some of the work, and tested additional compounds. These included pyrrole α-aldehyde, pyrrole α-carboxylic acid, disodium bilirubinate, mesoporphyrin IX, succinimide, and tryptophane. All the compounds were tested with corn, and in addition, the aldehyde was tested with barley and Chlorella.

The data reported here are limited by the low yields obtained in the laboratory synthesis of the pyrrole derivatives. These must be carefully purified to ensure that the observed effect is not ascribed to an accompanying impurity which has been inadvertently added. Furthermore, the results were so clear-cut, as to the toxicity or harmlessness of the compounds tested, that this brief note would seem warranted.

Experimentation

Golden Bantam corn, treated with 1 per cent. $HgCl_2$ and then 70 per cent. alcohol was germinated in the dark at 30° C. for 4 to 5 days, exposed to light for 2 to 3 days, and then transferred to aerated nutrient solutions (8) containing the essential elements except for iron. The time for almost complete chlorosis in the new leaves ranged from 5 days in the summer to 10 days in the winter. Sacramento barley was similarly germinated, and chlorosis was marked in from 4 to 7 days. *Chlorella vulgaris* was grown in a solution containing 1.5 per cent. glucose (purified by autoclaving with $CaCO_3$), 0.001 M KNO_3, K_2HPO_4, and $MgSO_4$. An air stream containing 5 per cent. CO_2 was passed through the culture. New cultures were grown as needed from 3 ml. of a 10-day-old suspension. Illumination was by means of 30-watt fluorescent lamps, 3 to 4 inches from the culture flasks.

The compounds tested were added in concentrations ranging from 0.025 to 0.250 gm. per liter. This dosage is from two-thirds to thirteen times the theoretical needed for synthesis of chlorophyll in the green controls.

Chlorophyll was estimated spectrophotometrically on acetone extracts of about sixty samples of homologous leaves in the different experiments, and the results for four of the compounds are summarized in table I.

In the case of the pyrrole aldehyde and carboxylic acid, the plants wilted, developing a purple color in the roots and leaves, and the degree of damage increased with increase in concentration. The aldehyde was more toxic than the acid, in which ten days was an average survival time, whereas the plants were dead in from 1 to 4 days with the former. Mesoporphyrin was also toxic, the plants showing severe wilt in 2 days, and dying within 5 days. Chlorophyll therefore was not determined.

To the Chlorella cultures there were added 32, 64, and 128 mg. of pyrrole aldehyde per liter. Purple discoloration developed within 48 hours at the higher concentrations, and after 72 hours at the low level.

TABLE I

CHLOROPHYLL CONTENT OF PLANTS WITH ADDED DERIVATIVES OR SUBSTITUTES FOR IRON

COMPOUND TESTED AND CROP	CHLOROTIC CONTROL	NO IRON + COMPOUND	IRON + COMPOUND	IRON CONTROL	REMARKS
Pyrrole aldehyde					Highly
(Corn).	1.00	1.26 ± 0.29*	†	4.53	toxic
(Barley)	1.00	1.17 ± 0.05	1.80 ± 0.05	2.30	"
Succinimide					
(Corn)	1.00	0.90 ± 0.06		4.55	Non toxic
Disodium bilirubinate					
(Corn)	1.00	1.00 ± 0.05	13.2 ± 0.1	35.0‡	"
Tryptophane					
(Corn)	1.00	1.00 ± 0.06		35.0	"

* The high value is due to the wilted moribund condition of the samples.
† The toxicity of the aldehyde was apparent even in the presence of four times the necessary amount of iron.
‡ The controls have high values as the non-toxic compounds could be tested over longer periods of time.

No bilirubinate was visibly absorbed by the roots. Disodium bilirubinate is normally oxidized to biliverdinate in 12 hours. This did not happen in the culture solution in 8 days.

The tryptophane and succinimide were Eastman Kodak (techn.). Tryptophane was used without further purification. Succinimide was twice recrystallized from acetone. The mesoporphyrin was prepared from hemin by reduction of hemin with HI and red phosphorus, and recrystallized from HCl. Bilirubin was extracted from gall stones of cattle, and was carefully purified.

The pyrrole carboxylic acid was prepared from pyrrole by the action of CO_2 on the Grignard pyrrole. It was recrystallized in H_2O.

The aldehyde was synthesized from pyrrole by the usual REIMER-THI-MANN method ($KOH + CHCl_3$). It was twice recrystallized from petroleum ether.

Summary

The theory that pyrrole derivatives may replace iron in the synthesis of chlorophyll by green plants has not been confirmed.

DIVISION OF FRUIT PRODUCTS
THE UNIVERSITY OF CALIFORNIA

LITERATURE CITED

1. DEMIDENKO, T. Iron in the nutrition of higher plants. Compt. Rend. Acad. Sci. **15**: 267–271. 1937.
2. DEUBER, C. Can a pyrrole be substituted for iron in the growth of plants? Amer. Jour. Bot. **13**: 276–285. 1926.
3. GODNEV, T. Rôle of iron in the formation of chlorophyll. Bull. Polytech. Iust. Ivanovno-Vosdnesensk **10**: 87–92. 1927.·
4. LODOLETTI, A. Influence of the pyrrole nucleus upon the formation of chlorophyll. Boll. Chim. Farm. **77**: 609–610. 1938.
5. POLLACCI, G. Influence of the pyrrole nucleus upon the formation of chlorophyll. Ber. d. bot. Ges. **53**: 540–542. 1935.
6. —————, and ODDO, B. Influence of the pyrrole nucleus upon the formation of chlorophyll. Rend. Accad. Lincei, **24**: 37–39. 1915. Gazz. Chim. Ital. **45**: 197–200. 1915.
7. —————, —————, and GALLOTTI, M. Influence of the pyrrole nucleus upon the formation of chlorophyll. Boll. Soc. Ital. Biol. Sper. **10**: 565–567. 1935.
8. STOUT, P., and ARNON, D. I. Experimental methods for the study of the rôle of copper, manganese and zinc in the nutrition of higher plants. Amer. Jour. Bot. **26**: 144–149. 1939.

LENGTHENING THE STORAGE LIFE OF APPLES BY REMOVAL OF VOLATILE MATERIALS FROM THE STORAGE ATMOSPHERE

F. W. SOUTHWICK AND R. M. SMOCK

McIntosh apples held in controlled atmosphere storage (*i.e.*, controlled oxygen and carbon dioxide levels) will keep two to three times as long as similar apples held in ordinary cold storage. When this technique is used, volatiles produced by the apples tend to accumulate in the gas tight chamber. Because of such accumulations the apple scald disease is likely to be severe unless shredded oiled paper is used in quantities around the fruit.

Attempts have been made to find a cheaper and more satisfactory control of this disease than the use of oiled papers. In an earlier report[1] it was noted that some promise was shown by various methods of removal of these volatiles by "air conditioning" the atmosphere. During the 1942–1943 season a semi-commercial trial with 1800 bushels of McIntosh apples was made of air conditioning the storage atmosphere with a highly refined oil. This technique controlled the scald on the more mature lots of fruit in storage; but on the greener lots there was four per cent. of scald. While this might have been considered commercial control, it is not perfect control. A semi-commercial trial of air conditioning the atmosphere of a 300-bushel controlled-atmosphere chamber full of McIntosh with activated lignite charcoal did not give commercial control of scald. While it controlled the disease on the mature lots of fruit, there was thirty per cent. of scald on the greener fruits.

In a series of trials with two-bushel lots of McIntosh apples in controlled-atmosphere storage, the only air conditioning agent that gave perfect control of scald was activated charcoal on which bromine had been adsorbed. Untreated activated charcoal did not give control. An oil wash for the atmosphere gave results comparable to those described in the semi-commercial test. Alkaline potassium permanganate did not give control.

A second result noted in the use of brominated charcoal was that the storage life of the apples was considerably lengthened (aside from scald control). Approximately two or three months was added to their storage life. Apparently enough ethylene is generated even in controlled atmosphere storage by the fruit, that its removal may retard ripening.

In laboratory trials it had been found that brominated charcoal was the only really effective agent in removing naturally evolved ethylene from rather ripe fruits. The effect of a rather ripe lot of apples in storage in stimulating the ripening of another less ripe lot of fruit in air storage (presumably through its evolution of ethylene) was eliminated by air conditioning the air with brominated charcoal.

It was found that activated lignite charcoal was not effective in controlling scald, although it seemed an excellent absorbent for artificially

[1] SMOCK, R. M., and SOUTHWICK, F. W. Some factors affecting the apple scald disease. Science n.s. 95: 576–577. 1942.

added esters. This would seem suggestive that esters were not the causative agent of scald. The fact that bromine is so effective in controlling scald suggests that scald is in some way related to an unsaturated hydrocarbon.

The production of volatiles by McIntosh apples was less in 1942–1943 than in the previous season—so the results reported may not be as favorable in a year of high volatile production and high incidence of scald.

Preliminary experiments indicate that it is unlikely that scald can be easily controlled on a very scald-susceptible variety like Rhode Island Greening by air conditioning the atmosphere with brominated charcoal, since excessively rapid air movement is necessary to accomplish control even by ventilation with fresh air.

DEPARTMENT OF POMOLOGY
CORNELL UNIVERSITY

NOTES

Annual Election.—In the annual election of 1943, Dr. JOHN W. SHIVE was elected as a member of the executive committee for a term of three years. A tie vote for a member of the editorial committee has been resolved by the executive committee in favor of Dr. E. J. KRAUS, who has been elected for a term of three years.

Life Membership Committee.—The CHARLES REID BARNES life membership committee has been appointed by the president, Dr. BERNARD S. MEYER, of Ohio State University. The chairman of the committee is Dr. R. B. HARVEY, Minnesota. The other members of the committee are Dr. R. E. GIRTON, Purdue; Dr. F. S. HOWLETT, Ohio State University; Dr. G. S. AVERY, Connecticut College; and Dr. F. WENT, California Institute of Technology. The 1943 award is the 20th, and is to be awarded to a distinguished plant physiologist from some foreign land, according to the provision of the by-laws that every fifth award is to be made outside the United States. The task of making the selection is always a pleasant one, but made more difficult than usual by war conditions. Announcement of the award may be expected in the January, 1944, issue of PLANT PHYSIOLOGY. The original announcements have always been a part of the annual dinner ceremonies, but there is at the present moment no prospect of a national annual meeting. If, however, any regional meeting of the A.A.A.S. or other organizations are arranged, notices of such meetings will be made public and all members informed.

Purdue Section.—The Purdue section of the American Society of Plant Physiologists maintains its activities with an interesting series of programs. The section meets twice each month in Room 103 Coulter Hall, at 4:00 P.M. The following titles are on the program for the first half of the current season:

October 18.—L. J. SWIFT: The viscosimetric determination of free menthol and peppermint oil.

November 15.—G. B. CUMMINS: Some defoliation studies on tomatoes.

December 6.—W. R. MULLISON: Some recent experiments in plant nutrition.

December 20.—C. L. PORTER: Penicillin to date.

January 3, 1944.—G. O. MOTT: The association of grasses and legumes in pasture mixtures.

January 17.—E. W. STARK: Comparative anatomy of woods.

Dr. LAURENZ GREENE, Department of Horticulture, is chairman of the Purdue section, and represents the Purdue section on the executive committee of the society.

New England Section.—The executive committee of the New England section voted last spring that it was not advisable to hold the regular meeting. It was also decided that the organization should remain *in statu quo* with regard to officers until officers could be elected at a meeting to be held under more normal conditions. The secretary, Dr. LINUS H. JONES, suggested that members prepare papers, since it seemed that graduate students would not be available.

Committee on the Editorship.—The approaching retirement from active service of the present editor-in-chief of PLANT PHYSIOLOGY has made it necessary to consider the appointment of a successor as of the end of 1944. To meet this situation, Dr. LOOMIS, during his term as president, named a committee to canvass the situation. The personnel of the committee is as follows: Chairman, Dr. O. F. CURTIS, Cornell University; Dr. F. P. CULLINAN, the U. S. Department of Agriculture; Dr. B. M. DUGGAR, the University of Wisconsin; Dr. GEORGE W. SCARTH, McGill University; and Dr. SAM TRELEASE, Columbia University.

The executive committee will, of course, have to make the final decision. In December, 1943, it will be two years since this matter first came up, and a prompt decision is desirable in order that the incoming editor-in-chief may become familiar with every phase of the editorial work during the remaining year in which cooperation will be possible. The cancellation of the annual meeting in 1942 prevented the usual meeting of the executive committee, and no opportunity for discussion of the problem has arisen. The editor-in-chief desires to cooperate with those who have the responsibility of making the selection, and will be happy to correspond with any members of either committee. Time is of the essence, and there is little time to lose.

Errata.—Attention of members and subscribers is called to the list of errata at the close of the table of contents of volume 18. We regret the errors, and suggest that owners of volumes enter the corrections so that at no future time could the errors cause other errors to be made. Thanks are accorded to those who kindly called attention to the mistakes. All of our authors are requested to take the same interest in their own papers, and to report to the editorial office anything that needs correction.

———

George Lincoln Teller.—In the death of GEORGE LINCOLN TELLER on November 13, 1942, the American Society of Plant Physiologists lost one of its older members. Mr. TELLER was a well-known figure in cereal chemistry and research. Since 1926 he had been president of the Columbus Laboratory in Chicago.

He was born at Colon, Michigan, January 23, 1867. His education was obtained in the public schools of Michigan, and at the Michigan State College, which granted him the B.S. degree in 1888, and M.S. in 1893. For two

years, 1888–1890, he was assistant chemist at the Experiment Station at East Lansing. In 1890 he was called to the Arkansas Station as chemist, a position which he held for nine years. He was connected with the Chidlow Institute in Chicago for several years, beginning in 1899; and in 1902 he began work with the Columbus Laboratory. In 1926 he began his term as president of the laboratory.

He was deeply interested in the chemistry of flour and other cereal products, and studied the proteins, mineral content, sugars, diastatic activity, etc. He was also a student of starch grain formation and structure. Two of his numerous papers were published in PLANT PHYSIOLOGY: one on changes in nitrogen compounds in the wheat grain at different stages of development, in 1935; the other on plant diastase in evidence as to the formation and structure of the starch granules, in 1938. In the Journal of Biological Chemistry for 1936 we note a paper entitled: Evidence concerning two types of diastase. He contributed a number of papers to Cereal Chemistry, and a series of bulletins from the Columbus Laboratory, reprinted from the Northwestern Miller, National Miller, American Food Journal, etc.

He was a man of dynamic personality, and contributed to the growth and development of the Columbus Laboratory into a fine testing and research organization. His contribution was a worthy one, a definite humanitarian service. He will be greatly missed by his colleagues and friends.

Mineral Deficiencies.—A very useful color atlas and guide to mineral deficiencies in plants has been published by the University of Bristol Agricultural and Horticultural Research Station at Long Ashton, Bristol. The work has been prepared by T. WALLACE, under the title: *The Diagnosis of Mineral Deficiencies in Plants by Visual Symptoms.*

There are five chapters of text, following the preface and introduction, titled as follows: Essential points in the nutrition of plants; soils in relation to the supply of mineral nutrients; methods of determining mineral deficiencies in crops; visual symptoms of deficiencies in crops; and use of the visual method of diagnosis in the field. The discussion is concise and yet adequate. The most important part of the work, of course, is the atlas of colored plates which are printed two to the page, but they are large enough to convey to the student the essential symptoms of deficiency. The total number of plates is 114, a very fine coverage. They are arranged into a number of groups: 1. Potatoes grown in sand cultures. 2. Natural autumn foliage tints. 3. Deficiencies as shown in field crops. The deficiencies illustrated are: phosphorus, calcium, magnesium, potassium, iron, manganese, and boron. 4. Symptoms which may be confused with natural deficiencies. These include virus injuries, insect damage, and chloride toxicity.

There are always difficulties in reproduction of colors for such works, and in this case the principal difficulty seems to be in shades of green, which seem to the reviewer to be somewhat too blue in some instances. On the

whole, however, it is a very valuable guide, and should prove extremely serviceable to the practical grower, as well as to the student who is trying to become familiar with troubles of deficiency at sight.

Many American laboratories and individual workers will want this valuable atlas, both for its text, and for its colored plates. The price is only 10/- net, and the orders should be addressed to H.M. Stationery Office, York House, Kingsway, London W.C.2.

Soil-Yield Theory.—This work, in German, discusses the problems of yield from the standpoint of mathmatical computation. It is entitled *Bodenertrags-Theorie,* and the author is Dr. KARL DÜRR. The introduction deals with the methods, and fundamentals of yield theory. The first chapter presents the scientific-technical considerations, plant physiological, and the application of yield laws to agriculture. The second chapter, with eleven sections, deals with the entire course of yield or production as an agricultural problem, and the economics of agricultural production. It takes up and discusses in thoroughly modern fashion the problems that confronted MALTHUS and JOHN STUART MILL . . . and the problems of gross receipts (Rohertrag), net receipts (Reinertrag), proceeds (Erlös), and expenses (Aufwand). The presentation necessarily involves mathematical equations, but these are readily understood by anyone who has given the matter some attention.

The book gives a very good summary of land yield and rental problems, based upon sound scientific principles. Eight text figures assist one in grasping the mathematical relations. It ought to be a valuable work for technical agronomists and plant physiologists, but would have to be interpreted in simple terms if it is to become available to the ordinary agriculturist. Agricultural economy is a complex problem, and few dirt farmers have any conception of the intricate economic relationships of production factors.

The book, with brief index, occupies only 96 pages. It is bound in paper. The publisher is Verlag Fr. Dürig. Ostermundigen-Bern, Switzerland. The price quoted is very reasonable, 4.5 Swiss francs. Orders should be sent to the publisher at the address given.

Cellulose and Cellulose Derivatives.—A compendious work dealing with cellulose and its derivatives has been prepared by a group of specialists under the editorship of Dr. EMIL OTT, Director of Research of the Hercules Powder Co., Wilmington, Delaware. It belongs in the series dealing with High Polymers, volume V in the series. There are several dozen contributors to the volume, and the contents are arranged into ten chapters with the following titles: Occurrence of cellulose; chemical nature of cellulose and its derivatives; structure and properties of cellulose fibers; carbohydrates normally associated with cellulose in nature; lignin and other noncarbohydrates; preparation of cellulose from its natural sources; bleaching

and purification of cellulose; derivatives of cellulose; physical properties of cellulose and its derivatives; and technical applications of the physical properties of cellulose and its derivatives.

The indexes are unusually full, and occupy 66 pages. The entire monograph contains 1176 pages, and its size of course makes it an expensive book to own. The price quoted is $15.00 per copy. Nevertheless, one cannot anywhere else obtain so full an account of cellulose, its nature and uses. The Interscience Publishers, Inc., deserve congratulations for the fine service they are rendering to scientific and industrial progress in the publishing of such books as *Cellulose and Cellulose Derivatives.*

Copies may be ordered from the publishers, Interscience Publishers, Inc., 215 Fourth Ave., New York, N. Y.

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[1] Papers published in PLANT PHYSIOLOGY are indexed in The Agricultural Index, The W. H. WILSON Co., New York.

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